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Prepared by

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AN INVESTIGATION OF CHEMISTRY AND TOXICITY OF OZONE-PRODUCED OXIDANTS AND BROMATE TO SELECTED ESTUARINE SPECIES

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

- the effects of toxic organic pollutants on individual species and communities of organisms;
- the effects of toxic organics on ecosystem processes and components;
- the significance of chemical carcinogens in the estuarine and marine environments.

Although widely used for disinfection of municipal and industrial wastewaters, chlorine and its residual by-products are known to be toxic to aquatic life. This report describes research to determine the effects of selected alternative oxidizing agents on estuarine organisms. Results of this research will be used to aid in the evaluation and selection of the most environmentally compatible disinfectant process under conditions existing for each application.

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ABSTRACT

This research program was initiated to determine the chemical and biological effects of ozone in estuarine systems and to evaluate its suitability as an alternative to chlorine. Chemical studies were performed to determine oxidant decay rates and bromate formation in both natural and artificial estuarine water. Ozonation of estuarine or marine waters can produce significant amounts of bromate if the natural levels of ammonia-nitrogen and other competing compounds are low and the initial residual oxidant and bromide concentrations are high. Toxicity studies showed that the concentrations of bromate which theoretically could be formed in an ozonated discharge were not toxic to the early life stages of striped bass (*Morone saxatilis*) and juvenile spot (*Leiostomus xanthurus*).

Acute toxicity studies of ozone-produced oxidants (OPO) showed that the early larval stages of the American oyster (*Crassostrea virginica*) and recently hatched striped bass ichthyoplankton were most sensitive to OPO; this sensitivity decreased with progressive development. Juvenile blue crabs (*Callinectes sapidus*), juvenile Atlantic menhaden (*Brevoortia tyrannus*) and adult white perch (*Morone americana*) were more resistant than molluscan and ichthyoplankton larval stages. Hematological and gill histopathological sublethal studies of adult white perch showed that OPO caused death by hypoxia as a result of excessive gill damage. Moderately damaged gill tissue repaired itself within a few days when fish were moved to non-ozonated water.

A comparison of the ozone data obtained in this study with similar chlorine data from the literature showed that the toxicity of ozonated and chlorinated estuarine water is similar. Thus, ozone appears to offer few, if any, advantages over chlorine in reducing toxicity to estuarine organisms.

This report was submitted in fulfillment of Grant No. R804683 originally awarded to the Academy of Natural Sciences of Philadelphia under the sponsorship of the U. S. Environmental Protection Agency. This report covers a period from August 30, 1977 to August 29, 1980; work was completed as of August 29, 1980.

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SECTION 1

INTRODUCTION

Chlorine has been widely used for disinfection of municipal and industrial wastewaters and the control of biological fouling in power stations. Several recent studies have shown, however, that chlorine and its residual byproducts are toxic to aquatic organisms (Brungs, 1973; Tsai, 1975; Whitehouse, 1975; Brungs, 1976; Davis and Middaugh, 1976; Morgan and Carpenter, 1978; Hall et al., in press a and b) and may also produce reaction products hazardous to man (Jolley, 1973; Glase et al., 1976; Jolley et al., 1978; Jolley et al., 1980). For these reasons several oxidizing agents, such as, bromine, bromine chloride, hydrogen peroxide and ozone, have been proposed as alternatives to chlorine (U. S. Environmental Protection Agency, 1976; Burton and Liden, 1978; Garey, 1980).

One of the most viable alternatives to chlorine for water and wastewater treatment is ozone (Evans, 1972; Blogoslawski and Rice, 1975; Fochtman et al., 1977). Numerous workers have shown that relatively low concentrations of ozone will kill microorganisms in water more effectively than chlorine (Venosa, 1972). Additional investigations have shown that ozone is a much more effective viral control agent than chlorine (Katzenelson and Shuval, 1975; Lockowitz et al., 1975; Sproul and Majumdar, 1975). Pilot plant studies and technical advancements in waste water treatment processes have also demonstrated that ozonation is competitive with chlorination from several standpoints and, therefore, should be considered a viable alternative to chlorine (Majumdar and Sproul, 1974; Ward et al., 1976; Ward et al., 1977).

The chemistry of both chlorine and ozone has been extensively studied in fresh water (White, 1972; Rice and Browning, 1975; Helz, in press). Less is known, however, about the chemistry of chlorine and ozone in marine and estuarine waters, which receive large quantities of chlorinated water from waste treatment facilities, power plants and other treated effluents. Chlorination or ozonation of coastal waters differs from similar treatment of fresh water in that Br^-, present in marine water, becomes oxidized to HOBr, OBr^- and bromamines (Eppley et al., 1976; Blogoslawski et al., 1976; Ingols, 1978; Williams et al., 1978; Helz and

Hsu, 1978; Helz et al., 1978; Kosak-Channing and Helz, 1979). Recent investigations have also shown that bromate, a stable, non-amperometrically detectable end-product may be produced following chlorination (Macalady et al., 1977) and ozonation (Crecelius, 1978 and 1979). Therefore, studies on the chemistry of bromate were conducted in this project in an attempt to clarify some of the poorly understood areas of oxidant reactions with saline waters. Because of the limited and extremely wide range of bromate toxicity data available in the literature (Crecelius, 1979; Stewart et al., 1979), bromate toxicity studies on the early life stage of striped bass (*Morone saxatilis*) and juvenile spot (*Leiostomus xanthurus*) were also conducted to supply much needed data in this area.

Ozone toxicity to fresh water organisms has been reasonably well documented (Hubbs, 1930; Arthur and Mount, 1975; Arthur et al., 1975; Rosenlund, 1975; Le Paulone, 1975; Ward and DeGraeve, 1978a, b and c; Wedemeyer et al., 1979; Paller and Heidinger, 1979; Asbury and Coler, 1980; Paller and Heidinger, 1980; Ward and DeGraeve, 1980). However, investigations on estuarine and/or marine organisms are limited (Toner and Brooks, 1975; Block et al., 1978). Therefore, the majority of this investigation dealt with the toxicity of ozone to the following estuarine species which were selected because of their commercial (economic), ecological and/or recreational importance. The following species and their respective stages were used to evaluate the toxicity of ozone:

American oyster (*Crassostrea virginica*) - larvae and adults
Blue crab (*Callinectes sapidus*) - juveniles
Striped bass - eggs, larvae and fingerlings
Atlantic menhaden (*Brevoortia tyrannus*) - juveniles
White perch (*Morone americana*) - adults

Studies were designed to meet the following objectives: 1) established acute dose-mortality relationships; 2) elucidate the physiological basis of acute toxicity; 3) examine physiological responses to sublethal levels of ozone; 4) compare the effects of ozone with those of previously published chlorine toxicity data and 5) evaluate the environmental acceptability of ozone as an alternative to chlorine. Various aspects of the chemical and toxicological studies discussed in this report have been published or accepted for publication. These include: Block et al. (1978), Helz et al. (1978), Richardson et al. (1978), Rosenkranz et al. (1978), Rhoderick et al. (1978), Richardson and Burton (1981), Hall et al. (in press c), Richardson et al. (in press a, b, c and with editor).

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

Oxidant decay in estuarine water was found to follow an approximate first order decay characterized by an initial fast component followed by a much slower component towards the end of decay. Light exposure accelerated the residual oxidant decay rates for both ozone-produced oxidants (OPO) and chlorine-produced oxidants (CPO) which in turn were inversely related to sample salinities in artificial sea water. Significant quantities of bromate were produced in light-exposed, chlorinated samples with an initial CPO concentration of ≈ 5 mg/L, but not at lower residual oxidant concentrations or in non-photolyzed samples. No bromate was formed in any of the chlorinated natural estuarine water samples. Bromate production was much greater in ozonated samples than in chlorinated samples and was formed in two distinct stages. Photolytic bromate formation decreased with increasing bromide concentration in both chlorinated and ozonated artificial sea water. Bromate formation was completely inhibited in the presence of NH_3-N and estuarine sediment. The same free radical mechanism is proposed for both ozone-induced and photolytic-induced bromate formation in artificial sea water.

Bromate toxicity studies conducted on the early life stages of striped bass (*Morone saxatilis*) and juvenile spot (*Leiostomus xanthurus*) showed that bromate was not very toxic to ichthyoplankton or juvenile fish. The most sensitive life stage was striped bass prolarvae (96-h LC50 = 30.8 mg BrO_3^-/L) followed by older larvae, eggs and juvenile spot. The present study and other laboratory studies suggest that the amount of bromate that could theoretically be formed in an ozonated or chlorinated estuarine or marine discharge would be considerably lower than that found to be toxic to our test species. Another study has shown, however, that American oyster (*Crassostrea virginica*) larvae may be an order of magnitude more sensitive than other invertebrates, ichthyoplankton, juvenile and adult fish. Therefore, we suggest that studies be conducted in the field to determine actual concentrations of bromate that may be produced in an ozonated or chlorinated system. If the concentrations approach those which may be detrimental to molluscan larvae, additional studies should be conducted to verify the toxic effects on oyster larvae.

Acute toxicity studies of OPO showed that the early larval stages of the American oyster (straight-hinge larvae 96-h LC50 = 0.12 mg OPO/L) and recently hatched striped bass ichthyoplankton (larvae 96-h LC50 = 0.08 mg OPO/L) were the most sensitive to OPO; sensitivity decreased with progressive development. Fresh water-adapted striped bass eggs (12- to 30-h LC50 = 0.05 mg OPO/L) were more sensitive than estuarine-adapted eggs (12- to 30-h LC50 = 0.21 mg OPO/L). Adult American oysters were very resistant to OPO, however, growth of oysters continually exposed to OPO concentrations $\geq$ 0.10 mg/L may be inhibited.

Juvenile blue crabs (*Callinectes sapidus*), juvenile Atlantic menhaden (*Brevoortia tyrannus*) and adult white perch (*Morone americana*) were more resistant (96-h LC50 values ranged from 0.20-0.36 mg OPO/L) than molluscan and ichthyoplankton larval stages. Adult white perch sublethal hematological studies showed that significant decreases in blood pH and increases in hematocrit occurred between OPO concentrations of 0.05 and 0.10 mg/L. Histopathological studies of the gills revealed minor changes at 0.01 mg OPO/L, moderate epithelial sloughing and increased mucous production at 0.05 mg OPO/L and extreme tissue damage at concentrations $\geq$ 0.10 mg/L. Moderately damaged gill tissue repaired itself within a few days when fish were moved to non-ozonated water.

A comparison of the ozone data obtained in this study with similar chlorine data showed that the toxicity of ozonated and chlorinated water is similar. Thus, the use of ozone appears to offer few, if any, advantages over chlorine in reducing toxicity to estuarine organisms.

SECTION 3

MATERIALS AND METHODS

GENERAL PROCEDURES

Aqueous solutions of ozone were produced by accurately metering small, concentrated quantities of ozonated estuarine water into larger quantities of clean oxidant-free estuarine water. Because of the extreme diversity of organisms and developmental stages tested, however, the absolute volumes of supply water and the size and type of experimental chambers were extremely variable. For this reason, specific details of the various test apparatus will be discussed in separate sections for each species.

Ozone-Produced Oxidant Production

Ozone gas was produced with an electrical discharge type ozonator (Model T-816, Welsbach Ozone Systems Corp., Philadelphia, PA), using dry (-40°C dew point) aviator oxygen. The ozone-oxygen mixture ($\approx 4\%$ O_3 by volume) from the ozonator was bubbled into the base of an 11.5-L, polycarbonate counter-current contact column (7 cm I.D.; 2.75 m high) at 1.5 L/min through a coarse porosity, 3-cm fritted glass disc. Temperature controlled Patuxent River water was pumped into the top of the column while ozonated water was drawn from the bottom and fed to glass rotometers through which it was metered into constant flow, calibrated siphons (Figure 1). These siphons supplied a constant flow of temperature controlled water from a head tank to the experimental chambers and were equipped with "Tees" through which ozonated water from the rotometers could be injected. OPO concentrations could thus be easily set, maintained or changed by adjusting the rotometers, altering the amount of ozonated water which was injected. One of the calibrated delivery tubes always supplied the control chamber and thus never received any ozonated water.

Experimental chambers and all surfaces exposed to ozonated water were constructed of oxidant-inert materials such as glass, teflon, silicone, polyvinyl chloride or polycarbonate. The desired experimental water temperature in the head tank was controlled by means of solenoid-regulated mixing of heated or chilled estuarine water. Depending upon

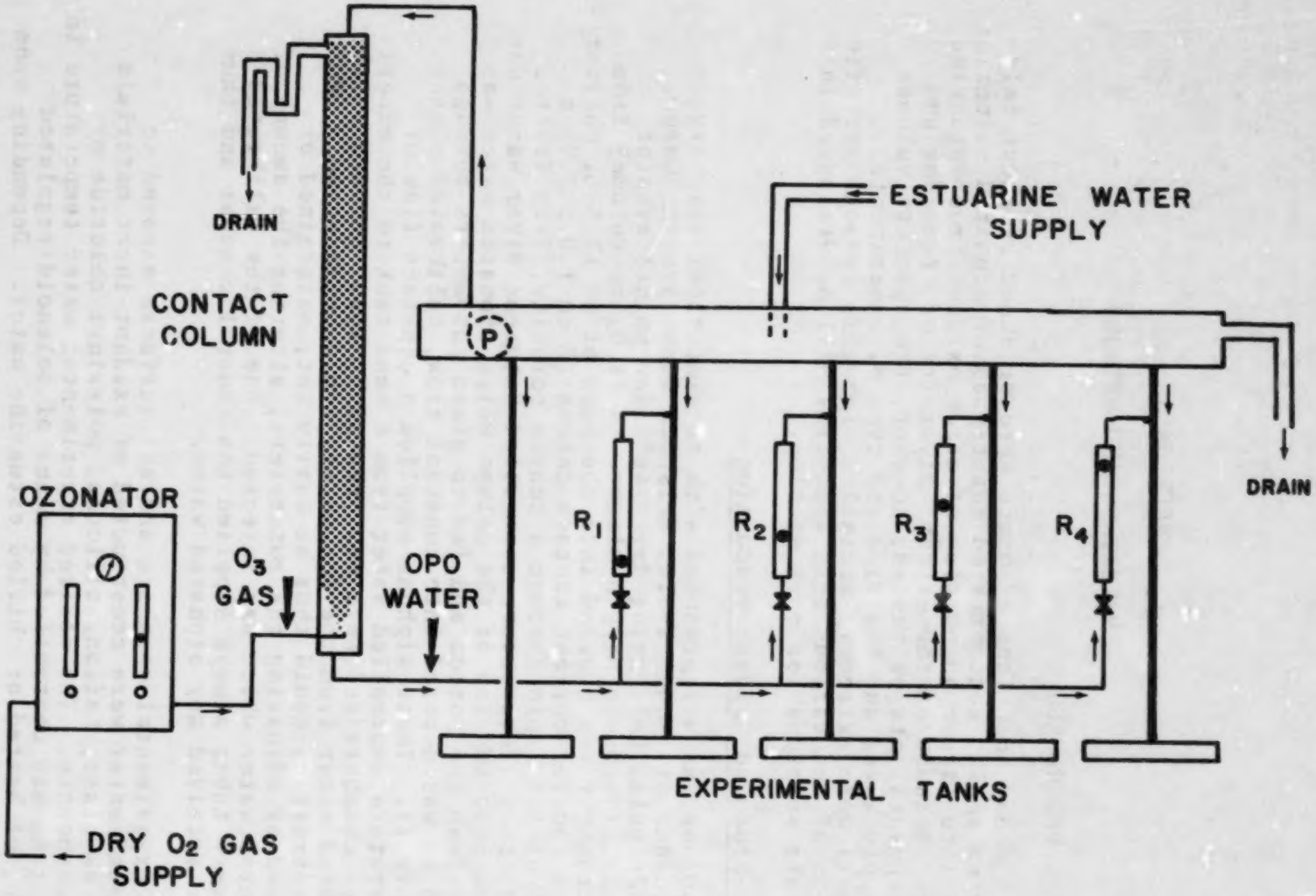
DRAIN
CONTACT COLUMN
OZONATOR
O_3 GAS
OPO WATER
DRY O_2 GAS SUPPLY
P
ESTUARINE WATER SUPPLY
R_1
R_2
R_3
R_4
DRAIN
EXPERIMENTAL TANKS

Figure 1. Schematic of the basic ozonation system. Flow rates and types of experimental tanks were dependent on the species and developmental stage being tested (OPO, ozone-produced oxidants; P, pump; R_1 to R_4, rotometers).

the water quality required for the various developmental stages, the water was filtered to various degrees during some experiments (e.g., larvae studies) and not in others (e.g., juvenile and adult fish).

Residual Oxidant Measurement

Ozone-produced oxidant (OPO) concentrations were measured amperometrically as equivalent total residual chlorine (American Public Health Association, 1976), using a PAR polarographic analyzer (Model 174A; Princeton Applied Research Corp., Princeton, NJ) equipped with a 10-g, rotating spiral platinum electrode (Englehart Industries, Rockville, MD) and a standard platinum disc reference electrode (Corning #476060). Phenylarsine oxide (2.82×10^{-3} N) was used as the titrant and was delivered with a syringe microburet (Model SB2; Micro-Metric Instrument Co., Cleveland, OH) capable of titrant additions as small as 3 µL. The potassium iodide and pH 4 reagents were prepared according to *Standard Methods* (American Public Health Association, 1976) and were premixed in the bottom of a 200 mL volumetric sample collection flask to minimize possible underestimation of the oxidant concentration (Carpenter et al., 1977; Crecelius et al., 1978). These procedures enabled accurate and reproducible OPO concentration measurements down to approximately 0.002 mg/L.

The terms ozone-produced oxidant (OPO) and chlorine-produced oxidant (CPO) are used throughout this report to refer to the amperometrically detectable total resident oxidant concentrations (measured as total residual chlorine) produced in solution following ozonation or chlorination, respectively. Thus, the term CPO is synonymous with the more commonly used terms total residual chlorine (TRC) and total residual oxidant (TRO).

Recent objections have been raised concerning the use of mg/L in reporting residual oxidant concentrations (Wong, 1980). However, mg/L has traditionally been used throughout most of the published literature dealing with ozone and chlorine and is currently the most widely accepted unit. Therefore, the primary unit of concentration used in this report will be mg/L. However, to avoid cases of ambiguity in the chemistry section, the mg/L value will be followed by the molar equivalent (i.e., µM) wherever confusion may occur.

CHEMISTRY STUDIES

Ozone-Produced Oxidant Decay

The rate of decay of OPO in estuarine and fresh water was studied to determine the persistance of these oxidants in each system since toxicity is a function of oxidant concentration and length of exposure. Decay in unfiltered Patuxent River estuarine water was studied for initial concentrations of 0.01, 0.10, 0.50 and 1.00 mg OPO/L at 15 and 25°C. The water quality at 15.2°C was: salinity, 12.2 ^{o}/oo; pH, 7.8 and no measureable NH_3-N. The water quality at 25.0°C was: salinity, 7.4 ^{o}/oo; pH, 7.5 and no measurable NH_3-N. Decay patterns in 17.0°C fresh well water (water hardness = 100 mg $CaCO_3$/L) were studied for initial OPO concentrations of 0.01, 0.05 and 0.10 mg/L. Standard fluorescent lighting was maintained during all studies.

The initial concentrations for each decay study were established by continuous-flow equilibration between ozonated and non-ozonated water inputs. The input equilibration volume used to establish each initial OPO concentration in both the 15°C river and 17°C fresh water studies was 12 L/min. However, only 6 L/min were available for the input equilibration volume in the decay study carried out in 25°C river water. In all cases a 46-L all glass aquarium was used as the decay tank for each concentration. Decays were initiated by removal of the input hoses after the selected concentration had been established. Concentrations were monitored frequently during the early phases of decay where decomposition rates were found to be very high and less often during the later phases where residual oxidants decayed more slowly.

Oxidant Decay and Bromate Formation

Decay rates and bromate formation were studied in three concentrations of artificial sea water as well as filtered estuarine water taken from the Patuxent River at Benedict, MD. Batches of artificial sea water were made up to salinities of 5, 15 and 30 ^{o}/oo, using double deionized -distilled water and commercially prepared artificial sea salt (Instant Ocean; Aquarium Systems, Mentor, OH). According to the manufacturer, this preparation contained no supplemental trace metals or organic materials such as amines, vitamins or culture nutrients. The total organic carbon content, while probably very low, was not measured. The NH_3-N concentration in all batches of artificial sea water never exceeded our minimum detection limit of 0.1 mg/L (5 μM). pH was adjusted to approximately 8.2, using Na_2CO_3 as needed. Salinity ranged from 4.0 to 6.5 ^{o}/oo, NH_3-N concentrations were < 0.1 mg/L (5 μM) and pH values were between 7.45 and 7.75 in the Patuxent River estuarine water.

Three residual oxidant concentrations were tested for each oxidant (ozone or chlorine) in each artificial sea water concentration (5, 15 and 30 ^{o}/oo) as well as the filtered estuarine water. Chlorine concentrations were obtained by addition of a concentrated $Ca(ClO)_2$ solution to 4 L of each test solution in an amount sufficient to produce initial (≈ 30 s after chlorination) residual oxidant concentrations of 0.1, 1.0 and 5.0 mg/L (1.4, 14.1 and 70.5 μM). It was necessary to add slightly more hypochlorite solution to the higher salinity samples to achieve equal initial oxidant concentrations. Ozone concentrations were produced by bubbling ozonated oxygen through a 4-L test solution with a frittered glass dispersion tube for a time sufficient to obtain initial (≈ 30 s after ozonation) residual oxidant concentrations of 1.0, 2.0 and 5.0 mg/L (14.1, 28.2, and 70.5 μM).

The contact time required to achieve a given initial residual oxidant concentration was lower in solutions containing higher Br^- concentrations or higher salinities compared to more dilute solutions. Figure 2 shows the amount of bromate formed and the time required to produce a measured residual ≈ 2.0 mg/L as TRO (28 μM) at different ozonator potentials in 5 ^{o}/oo artificial sea water. Because the ozonator voltage is proportional to the gaseous ozone concentration, the voltage can be used as a relative indicator of the ozone concentration, which is approximately 5.1 and 0.1% by weight at 116 and 64 volts, respectively. It can be seen that the curve for bromate formation increases logarithmically with the contact time and tends to level off at the longer contact times since the concentration of ozone in the gas phase is rapidly becoming smaller. If no residual oxidant limits were imposed, and exhaustive ozonation were carried out with a high ozone gas concentration, the bromate formation curve would be expected to increase steadily with time until all of the bromide present was converted to bromate as demonstrated by Crecelius (1978). Each 4-L test sample (chlorine or ozone) was split into two, 2-L subsamples. One subsample was placed into a 20°C water bath in complete darkness while the other was placed into a 20°C water bath illuminated by a bank of three, General Electric 275 watt R.S. sunlamps. Light and dark samples were then monitored over the next 24 h for both residual oxidant concentration and bromate formation.

The spectral distribution supplied by the General Electric Co. for the sunlamps is shown in Table 1. About 43% of the lamps total radiant power occurs in three bands of the near or very near ultraviolet region with the strongest band (≈ 33% of total radiant power) falling between 360 and 370 nm. Light intensity at the surface of the test solutions was measured at 1.07×10^3 lux (full, mid-day sunlight was measured at 2.01×10^4 lux), using a Quantum/Radiometer/Photometer (Model LI-185; Lambda Instrument Co., Lincoln, NE). The spectral response range of this meter was between 400

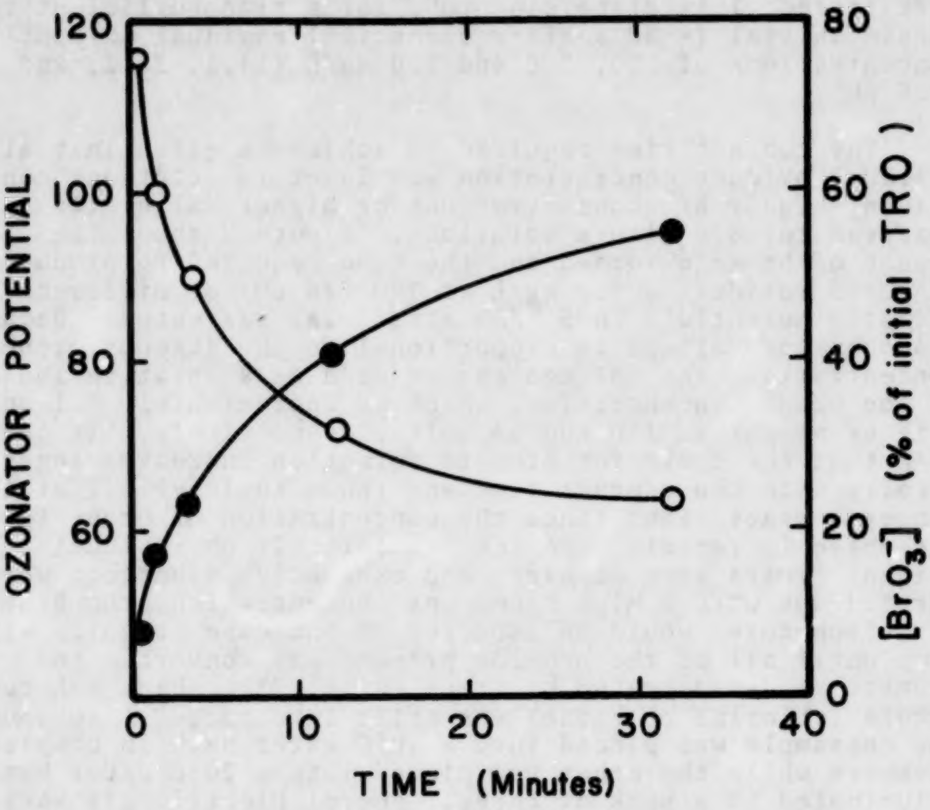

Figure 2. Bromate producation (●) and contact time required to produce ≈ 2 mg/L (28 μM) residual oxidant concentration for various ozonator potentials (O).

and 700 nm and thus did not respond to any of the ultraviolet radiation. It was extremely useful, however, in assuring a constant, reproduceable light intensity at the surface of each test solution.

TABLE 1. SPECTRAL DISTRIBUTION OF SUNLAMPS USED TO IRRADIATE CHLORINATED AND OZONATED SAMPLES

Radiant Power*	Wavelength Bond (nm)	Color Region
1.13	297.5-307.5	near ultraviolet
3.50	310.0-320.0	near ultraviolet
9.50	260.0-370.0	near ultraviolet
2.50	400.0-410.0	violet
4.50	425.5-435.5	blue
5.25	540.0-550.0	green
6.50	572.5-582.5	yellow

*Milliwatts/10nm/watt input.

Bromate concentrations were measured using differential pulse polarography on a PAR polarographic analyzer (Model 174A; Princeton Applied Research Corp., Princeton, NJ) connected to an X-Y recorder (Model 7040A; Hewlett-Packard, San Diego, CA). All bromate samples (15 mL) were placed into a constant-temperature, water-jacketed cell, maintained at 20°C and equipped with a PAR dropping mercury electrode (Model 174/70), a counting electrode and a Vycor tip, saturated, calomel reference electrode. Samples were then deoxygenated by vigorous bubbling with high purity nitrogen and polarograms recorded, using the following parameters: drop time 0.5 s, scan rate +2mV/s and an initial potential of -1.25 volts. The bromate peak potential and amplitude were affected by salinity, temperature and pH as follows. Salinities of 5, 15 and 30 ^{o}/oo resulted in corresponding peak potentials of -1.69, -1.67 and -1.65 volts, respectively, with no change in amplitude. Sample temperature was directly related to the bromate peak amplitude, which had no effect on peak potential (this source of error was eliminated by the constant temperature water jacket). Sample pH had no significant effect on peak amplitude or potential between pH 6.6 and 10.2 and thus was not of importance in our analyses.

However, below a pH = 6.5 peak amplitude rapidly increased, with the peak amplitude at pH 3.8 being over four times the amplitude at pH 6.6. Using these analytical procedures and equipment, bromate concentrations as low as 0.13 to 0.26 mg/L as BrO_3^- (1 to 2 μM) could be accurately detected.

After completion of the above study, it was noted that bromate formation and residual oxidant decay rates were markedly different for all three salinities of the artificial sea water samples, as well as the estuarine water samples, for both chlorine and ozone. In an effort to account for these differences, the effects of various concentrations of organic and inorganic substances on bromate formation and oxidant decay were briefly investigated. Chlorine was used as the oxidant in all cases and sample solutions were initially chlorinated to approximately 5 mg/L (71 μM) at 20°C. The compounds investigated were NH_3-N (1.5 mg/L as NH_3; 88.1 μM), 200 mg Patuxent River sediment/L (wet weight) and 50 mg KBr/L as Br^- (625.7 μM). Following initial chlorination, the extent of oxidant decay and bromate formation was measured after 6-h exposure under the sunlamps. Based on the results of this investigation further concentrations of KBr were investigated to determine the extent to which this substance appeared to inhibit bromate formation. Details of this analysis are fully covered in the results and discussion section.

TOXICITY STUDIES

All OPO exposure studies were performed under continuous-flow conditions, using minor modifications of the ozone contacting and delivery system previously described (Figure 1). Bromate toxicity experiments, however, were performed with static-replacement techniques since available quantities of sodium bromate were limited.

All acute toxicity data were analyzed by predictive mortality modeling techniques wherever possible. In these analyses, percentage mortality (Y) is expressed as a function of oxidant concentration and exposure time. Three transformations (log, logit and arcsine) were originally tested, using the stepwise procedure of SAS (Helwig and Council, 1979). The arcsine transformation was selected, however, since it consistently yielded the simplest models with the highest degree of accuracy. The following linear model was initially introduced to the stepwise procedure for this transformation for each set of mortality data:

$$\text{Arcsine}\,[(r/n)^{0\cdot5}] = a + b(C) + c(T) + d(D) + e(C^2) + f(T^2) + g(DC) + h(DT) + i(D^2) + j(\log C) + k(\log T)$$

where

C = exposure concentration (mg/L)

T = exposure time (h)

D = dose (C x T)

r = mortality response at a given dose

n = sample size at a given dose

a-k = coefficients estimated by the least squares procedure

Final models were selected based on the significance ($p < 0.05$) of a variable's contribution and simplicity of the equation.

Response surfaces were constructed from the predictive equations by back-transforming the predicted responses (Y), using the following equation:

$$\text{Predicted percentage mortality} = (\sin Y)^2 \times 100$$

Bromate

Bromate toxicity studies were conducted to determine the toxicity of bromate to estuarine ichthyoplankton and juvenile fish. Striped bass (*Morone saxatilis*) eggs-prolarvae and larvae and juvenile spot (*Leiostomus xanthurus*) were studied. Bromate concentrations were analyzed by differential pulse polarography, as described previously. The average water quality (± SD) during acclimation and testing for all studies was: temperature, 20.1 (± 0.3); salinity, 5.1 (± 0.1) °/oo; pH, 7.8 (± 0.0), dissolved oxygen, 7.0 (± 0.1) mg/L and NH_3-N, 1.0 (± 0.1) mg/L.

Striped Bass--

Fertilized striped bass eggs (water hardened) were obtained from the Brookneal Striped Bass Hatchery (Brookneal, VA) and transported to the laboratory in oxygen filled plastic bags, which were placed in styrofoam chests to maintain a constant temperature of ≈ 20°C. Eggs were transferred to standard 6-L hatchery jars supplied with a continuous-flow of filtered Patuxent River water (≈ 20°C); all dead and unfertilized eggs were allowed to purge from the stock jars. Larvae were reared in the laboratory from eggs under continuous-flow conditions, using filtered Patuxent River water. Prolarvae were fed *Artemia salinia* nauplii each day once they began active feeding (4-6 d post-hatch). Experiments were conducted with eggs which were 12 h old (measured from time of fertilization) and larvae which were 96 h old (post-hatch), using standard, renewal static bioassay procedures as outlined in *Standard Methods* (American Public Health

Association, 1976).

Experimental procedures--Forty striped bass eggs were placed in 1-L beakers containing bromate concentrations of 0, 10, 20, 50, 75, 100, 200, 300 and 500 mg BrO_3^-/L (as BrO_3^-). Test solutions, which were made using filtered Patuxent River water and reagent grade sodium bromate, were changed every 24 h. Controlled, artificial oxygenation was performed continuously throughout the duration of the egg and larvae studies to ensure adequate dissolved oxygen.

Eggs were continuously exposed to bromate for a period of 96 h. Eggs were allowed to develop through hatch, which occurred approximately 26 h into the study, (38 h post-fertilization); an estimation of percent hatch was made after 36 h (48 h post-fertilization) exposure. Total egg and/or prolarvae mortality was assessed at each concentration following 24, 36, 58, 72 and 96 h of exposure. Thus, the egg stage received approximately 26 h exposure to the bromate concentrations while prolarvae received a total of approximately 70 h exposure.

Twenty larvae were tested in each of nine, 1-L beakers containing the same bromate concentrations used in the egg study. Mortality observations were made every 24 h for 10 d; bromate solutions were changed every 24 h. Larvae were fed fresh *Artemia salinia* nauplii once each day throughout the 10-d test period. The larvae were maintained at approximately 20°C under a 12 h light: 12 h dark photoperiod.

Statistical analyses--Data for all experiments were analyzed using a SAS computer probit regression program (Helwig and Council, 1979). Probit regressions were performed stepwise for each exposure period and combined graphically to yield mortality x concentration x time response surfaces. A test of the goodness of fit for the response surfaces was derived by combining the individual chi-square values from each probit regression, as allowed by the reproductive property of the chi-square distribution (Beyer, 1971). During the egg-prolarvae study, the hatchability of the striped bass eggs was assessed by computing the percentage of successfully hatched eggs for each bromate concentration approximately 36 h into the experiment (48 h post-fertilization). For a complete discussion of the probit techniques used in these analyses, see Finney (1971).

Spot--

Juvenile spot were collected at Baltimore Gas and Electric Company's Calvert Cliffs Nuclear Power Plant (Calvert Cliffs), located on the Chesapeake Bay in Calvert County, MD. Fish were transported to the laboratory, where they were acclimated for at least 4 weeks in 800-L fiberglass holding tanks supplied with a continuous flow of Patuxent River water. All fish were fed twice daily with pre-cooked commercial shrimp and exposed to a 12 h light: 12 h dark photoperiod during acclimation and testing.

Experimental procedures--Groups of 15 spot, 92.9 (± 1.8) mm TL and 7.9 (± 0.6) g wet weight were placed in standard 46-L glass aquaria containing bromate solutions of 50, 75, 100, 180, 240, 320, 385, 470, 560 and 1000 mg BrO_3^-/L for 10 d. Mortality observations and water quality measurements were made every 12 h, while test solutions were changed every 24 h. Controlled, artificial oxygenation was performed continuously throughout the study.

Statistical analyses--The spot data were analyzed as described above for the striped bass studies.

Ozone-Produced Oxidants

American Oyster--

The American oyster (*Crassostrea virginica*) was selected since it is a commercially important species and because its life cycle includes both planktonic larvae and a sessile benthic adult. The acute toxic effects of OPO were evaluated for the straight-hinge and setting pediveliger larval stages. Shell deposition and fecal matter accumulation were used to evaluate the effects of OPO on adults because they have been shown to be sensitive physiological indicators of oyster condition (Scott and Middaugh, 1978; Rhoderick et al., 1977; Liden et al., 1980).

Larvae (General)--Adult American oysters, 2 to 6 years old, were obtained from the Wilde Oyster Hatchery, West River, MD. Oysters were scrubbed clean and placed in 1.2 x 2.4 x 0.2 m holding trays supplied with a continuous flow of 12°C filtered Patuxent River water (≈ 12°/oo). Over a 6-week period, the temperature was gradually increased from 12 to 20°C; oysters were fed daily with *Isochrysis galbana*. Spawning was induced by a sudden increase in both temperature and food supply, as described by Loosanoff and Davis (1963). Spawning oysters were removed from the spawning tray, sorted by sex and the sperm and eggs collected in separate tanks. Fertilization was initiated by mixing sperm and eggs and allowing them to remain in contact for 10 to 15 min. The end of this contact period was considered the developmental beginning (i.e., time 0).

After screening and washing, the density of the fertilized eggs was adjusted to 100/mL by adding 1-µ filtered Patuxent River water (≈ 12°/oo). During the first 48 h following fertilization, larvae had reached the early straight-hinge stage of development. Larval concentration was again adjusted to 100/mL and aliquots were pipetted into experimental chambers for testing. The remaining larvae in the stock culture were screened and washed through a 45-μ mesh screen, transferred to 19 L of 25°C, 5-μ filtered estuarine water (≈ 12°/oo), fed with *Isochrysis galbana* and then treated with 200 mg/L of Sulmet antibiotic. Screening, water changing, feeding and addition of antibiotics were performed on a daily basis for the stock culture.

Larval density decreased from ≈ 100/mL to ≈ 10/mL during the 9 to 21 d required for larvae to reach the pediveliger stage of development. When most of the pediveliger larvae had developed an eye spot and had begun setting, they were concentrated to 100/mL and transferred to a shallow setting tray. The tray was lined with black polyethylene sheeting and the entire bottom covered with numbered 2.5 x 7.6 cm glass microscope slides which were used as experimental substrates. When the density of setting pediveliger larvae reached 100 to 200/slide, each slide was removed and counted for the total number of live and dead larvae before being placed into an experimental chamber. Most of the setting pediveliger larvae were in the late crawling stage; very few were permanently cemented (Galtsoff, 1964; Cranfield, 1973). No larvae had reached the metamorphic stage of development at the beginning of the exposure period although some did undergo metamorphosis by the end of the exposure period.

Larvae (Experimental procedures)--Filtered (5 μ) Patuxent River water was used in all larval experiments to prevent clogging of the fine mesh on the bottoms of the experimental chambers. Filtered water from a head tank was delivered to each of five, 1-L distribution tanks through a 0.6 cm I.D. glass tubing. Each 1-L distribution tank was equipped with 10 supply lines, each of which supplied a continuous flow (≈ 20 mL/min) of test solution to a separate experimental chamber. Experimental chambers were constructed from 20 cm lengths of 7 cm I.D. glass tube with 35 μ nitex mesh attached to one end with silicone aquarium sealer. All 10 chambers at each concentration were placed in a 10-cm deep fiberglass tray to maintain an adequate water level (≈ 5 cm) in the chambers.

At the beginning of the straight-hinge larval experiment, 2 mL of the 100 larvae/mL stock culture were pipetted into each of the experimental and control chambers, yielding a total ≈ 200 larvae/chamber (≈ 1 larvae/mL test solution). Ten chambers were simultaneously started at each concentration of 0.000, 0.036 (±0.013), 0.098 (±0.024), 0.184 (±0.047) and 0.318 (±0.033) mg OPO/L. Selection of concentrations was based upon the results of preliminary range-finding experiments conducted with larvae from a previous spawn. Duplicates (one pair) were removed from each concentration after 12, 24, 48 and 96 h of exposure. Upon removal, chambers were placed in a bath of 0.1% neutral red dye for 15 min, then rinsed and washed through a 10-µ mesh collecting cup and placed in a Sedgewick-Rafter cell where mortality counts were made for each chamber under 300x magnification.

The neutral red dye acted as a "vital stain" and was picked up by the tissues of living larvae, causing them to appear pink and thus aiding in the determination of live or dead larvae. Additional death criteria were empty shells, caused by bacterial action, and lack of movement. Water quality during the straight-hinge larvae experiments was: temperature, 25.0 (±0.1)°C; salinity 11.9 (±0.3)°/oo; pH, 7.8 (±0.0) and dissolved oxygen which ranged from 7.4 to 11.6 mg/L for nominal OPO concentrations of 0.0 to 0.3 mg/L, respectively.

In the setting pediveliger larval experiments, glass microscope slides with approximately 200 larvae attached to one side were obtained from the setting tray described earlier. After counting, to determine the number of live and dead larvae, each slide was placed into an experimental chamber identical to those used in the straight-hinge study. Ten chambers were placed in each OPO concentration of 0.000, 0.061 (±0.024), 0.105 (±0.048), 0.180 (±0.055) and 0.277 (±0.064) mg/L; duplicates were removed from each concentration after 12, 24, 48 and 96 h of exposure to determine mortality. Concentrations and exposure times were selected to cover the same ranges used in the straight-hinge experiments. All slides that had been removed at each exposure time were placed in a staining bath of 0.1% neutral red dye for 15 min, then examined under 300x magnification to determine the total number of live and dead larvae. Criteria for death were the same as for the straight-hinge larvae with the exception of movement. Water quality during the pediveliger study was: temperature, 25.0 (±0.1)°C, salinity 11.9 (±1.0)°/oo; pH, 7.4 (±0.1), and dissolved oxygen which ranged from 7.8 to 11.1 mg/L for nominal OPO concentrations of 0.0 to 0.3 mg/L, respectively.

Larvae (Statistical analyses)--Predictive percentage mortality models for both straight-hinge and setting pediveliger larvae were derived as described previously. A comparison was made between the models to determine if there was a difference in the resistance of the two developmental stages to OPO. The difference between the relative resistance of straight-hinge and setting pediveliger larvae to OPO and CPO was also tested by comparing our models with similar models obtained from the literature (Roosenberg et al., 1980). All comparisons were performed with standard analysis of variance procedures (Sokal and Rohlf, 1969).

Adults (General)--Cultchless, 1- to 4-year old American oysters were obtained from the Wilde Oyster Hatchery, West River, MD and acclimated to either 15 or 25°C in large holding trays (1.5 x 3.0 x 0.2 m) supplied with continuously flowing Patuxent River water (≈ 9 °/oo) for 3 weeks prior to experimentation. These acclimation temperatures were selected as representative temperatures near the high and low limits encountered during the seasonal periods in which antifouling chlorination is conducted. During acclimation, American oysters were scrubbed clean at least once a week and fed daily with *Isochrysis galbana*. Production of various OPO concentrations was similar to the method used in the larval experiments. However, the Patuxent River water was not filtered, flow rates were much greater (200 mL/min) and 7-L fiberglass hatchery trays (Heath Tecna Corp., Kent, WA) were used as experimental chambers.

Adults (Experimental procedures)--Groups of 12 oysters (mean length = 7.5 ± 0.8 cm), acclimated to 25°C, were exposed to each OPO concentration of 0.000, 0.009 (±0.000), 0.046 (±0.002), 0.094 (±0.004), 0.174 (±0.006), 0.301 (±0.027) and 0.483 (±0.022) mg/L for two consecutive 6-d periods. The mean water quality was: temperature, 24.5 (±0.7)°C; salinity, 12.0 (±0.5) °/oo; pH, 7.9 (±0.2) and dissolved oxygen which ranged from 6.8 to 12.8 mg/L for nominal OPO concentrations of 0.0 to 0.5 mg/L, respectively. Shell deposition and fecal accumulation were measured twice following 6 and 12 d of exposure. A paired t-test showed no significant ($p > 0.05$) difference in either the shell deposition or fecal accumulation responses between the first and second 6-d periods of exposure.

Shell deposition was measured according to *Standard Methods* (American Public Health Association, 1976). Fecal accumulation was measured as a total accumulation for all oysters at each concentration and was quantified in the following manner. At the termination of an experiment, all accumulated fecal matter (feces and pseudofeces) was transferred to a large plastic container after removing the oysters and allowed to settle for 48 h. The supernatant was then siphoned off, the precipitate concentrated in a glass container and the volume adjusted to exactly 4 L. After resuspension, a 100 mL aliquot was removed and filtered through preweighed #2 Wattman filter paper. It was then dried at 100°C for 24 h and reweighed to obtain a fecal accumulation coefficient for each OPO concentration.

Groups of 10 oysters, acclimated to 15°C, were exposed to each OPO concentration of 0.000, 0.012 (±0.002), 0.052 (±0.016), 0.105 (±0.037), 0.205 (±0.047), 0.293 (±0.077) and 0.490 (±0.166) mg/L for 120 h. Because there was no difference in the response parameters at 25°C between the first and second 6-d periods of exposure, the study at 15°C was planned for only one 6-d period. Unfortunately, a power failure occurred during the night between the 120- and 132-h observation period. As adequate time and oysters were not available to repeat the experiment, shell deposition and fecal accumulation were measured following the 120-h exposure. Oysters tested had a mean length of 6.7 (±0.7) cm. Water quality was: temperature, 15.6 (±0.4)°C; salinity, 9.2 (±0.5) °/oo; pH, 8.0 (±0.2) and dissolved oxygen which ranged from 9.6 to 14.6 mg/L for nominal OPO concentrations of 0.0 to 0.5 mg/L, respectively.

Adults (Statistical analyses)--Shell deposition was analyzed at each acclimation temperature with an analysis of variance and Duncan's multiple range test, while fecal accumulation was analyzed with regression techniques. The mean shell deposition and fecal accumulation values for the first and second 6-d periods were utilized for the 25°C experiment since there was no significant difference. For comparisons between the two acclimation temperatures (15 and 25°C), it was necessary to normalize the data since the results at 25°C were for a 6-d test while results at 15°C were for a 5-d test. Therefore, the response parameters at 15 and 25°C were divided by their respective exposure periods (5 and 6 d) and then multiplied by 4 to get a normalized response based on a standard 96-h exposure. Differences between these normalized response parameters at the two acclimation temperatures were tested with a standard t-test.

Blue Crab--

Juvenile blue crabs (*Callinectes sapidus*) were used to evaluate the acute effects of OPO on a representative important recreational and commercial crustacean. Juvenile blue crabs were collected from the Chesapeake Bay near Calvert Cliffs using small dip nets and returned to the laboratory in large plastic tubs. At the laboratory, crabs were held in large 800-L fiberglass tanks supplied with a continuous flow of 25°C filtered Patuxent River water for at least two weeks prior to experimentation. During acclimation, crabs were fed cooked, commercially available shrimp supplimented with small amounts of frozen fish. Mortality during the acclimation period was minimal. The photoperiod during acclimation and testing was 16 h light: 8 h dark.

Experimental procedures--Twenty-five crabs (27.3 ± 8.7 mm; 2.3 ± 2.1 g) were continuously exposed to each OPO concentration of 0.000, 0 113 (± 0.031), 0.232 (± 0.031); 0.303 (± 0.037), 0.397 (± 0.078), 0.529 (± 0.040), 0.628 (± 0.088) and 0.674 (± 0.208) mg/L for 144 h (6 d) at a flow rate of 2 L/min in 56 L all glass aquaria. Mortality observations were made after 6, 8, 12, 18, 24, 36, 48, 60, 72, 96, 120 and 144 h exposure. Crabs were not fed during the experiments; cannibalism was minimized by placing clean [illegible] bricks, shells and similar substrate material in the tanks to provide adequate cover for molting crabs. Mean water quality during acclimation and testing was: temperature, 25.2 (± 0.4)°C; salinity, 7.4 (±0.3) °/oo; pH, 7.7 (±0.3) and dissolved oxygen which ranged from 4.5 to 10.0 mg/L for nominal OPO concentrations of 0.0 to 0.7 mg/L, respectively.

Statistical analyses--In addition to the percentage mortality model described previously, a SAS probit analysis (Helwig and Council, 1979) was used to calculate LC50 values for which 95% confidence limits could be calculated and thus compared quantitatively with the literature.

Striped Bass--

The acute effects of OPO on the early life stages of striped bass were evaluated in these studies. Water hardened fertilized eggs were obtained from Brookneal Striped Bass Hatchery in Brookneal, VA and transferred to the laboratory in oxygen filled plastic bags at approximately 18°C. Eggs were tested in both fresh and estuarine water because striped bass typically spawn in fresh water while the developing eggs and prolarvae are exposed to increasing salinities as they are transported down the estuary. Eggs were maintained in 12-L hatchery jars under continuous flow conditions. Larvae were reared in estuarine water (3.6 °/oo) from the egg stock to a total length of approximately 20 mm in hatchery trays at densities < 1 g/L. Larvae were fed natural plankton introduced via the seawater system and supplemented with high densities of brine shrimp, *Artemia salinia*. Fingerlings (≈ 13 cm total length), obtained from Ichthyological Associates Incorporated in Middletown, DE, were acclimated for 14 d before testing. These organisms were fed pre-cooked commercial shrimp twice daily during the acclimation period. Photoperiods for the acclimation and testing of all life stages were maintained at natural light-dark conditions which were approximately 12 h light: 12 h dark for eggs and larvae and 14 h light: 10 h dark for fingerlings. The mean water quality during each study is listed below in the appropriate experimental procedure sections.

Egg studies (Experimental procedures)--Three sets of 30 12-h old fresh water acclimated striped bass eggs were exposed to each concentration of 0.000, 0.011 (± 0.005), 0.043 (± 0.014), 0.070 (± 0.015) and 0.096 (± 0.014) mg/L. Three sets of 30 12-h old estuarine acclimated eggs (≈ 3.6 °/oo) were exposed to 0.000, 0.005 (± 0.001), 0.012 (± 0.003), 0.050 (± 0.010) and 0.100 (± 0.020) mg OPO/L. Eggs in both fresh and estuarine water were exposed to OPO concentrations for periods of 12 h, 30 h and approximately 36 h (hatch occurred when eggs were approximately 48 h old). All eggs were tested in PVC test chambers (volume 206 mL) fitted with 350-μm nylon mesh placed in 7-L hatchery trays supplied with ozonated water at 4 L/min. Mortality observations were recorded at the end of each exposure period. The criteria for death were a change from a normal transparent color to an opaque color and/or failure to hatch. The mean water quality for the fresh water eggs was: temperature, 16.4 (± 0.2)°C; pH, 7.8 (± 0.0) and dissolved oxygen which ranged from 5.9 to 9.1 for nominal OPO concentrations of 0.0 to 0.1 mg/L, respectively. The mean water quality for the estuarine eggs was: temperature, 17.5 (± 0.4); salinity, 3.6 (± 0.3) °/oo; pH, 7.6 (± 0.0) and dissolved oxygen which ranged from 7.9 to 9.0 for nominal OPO concentrations of 0.0 to 0.1 mg/L, respectively.

Larvae studies (Experimental procedures)--Striped bass larvae were subjected to continuous OPO concentrations of 0.000, 0.005 (± 0.001), 0.010 (± 0.003), 0.052 (± 0.005), 0.099 (± 0.008), 0.148 (± 0.008), 0.194 (± 0.010) and 0.310 (± 0.016) mg/L for periods up to 96 h. Forty larvae were tested at each concentration in 7-L hatchery trays supplied with 4 L/min ozonated water. Mortality observations, water quality and OPO concentrations were recorded at 6, 12, 24, 36, 48, 60, 72, 84 and 96 h. The criteria used for death of the larvae were cessation of opercular movement and/or lack of movement in response to a mild mechanical stimulus. The average water quality during acclimation and testing was; temperature, 23.0 (± 0.1), salinity, 11.2 (± 0.1) °/oo; pH, 7.7 (± 0.1) and dissolved oxygen which ranged from 8.0 to 11.0 mg/L for nominal OPO concentrations of 0.0 to 0.3 mg/L.

Fingerling studies (Experimental procedures)--Ozone-produced oxidant effects on striped bass fingerlings were assessed by continuously exposing test organisms to OPO concentrations of 0.000, 0.020 (± 0.004), 0.041 (± 0.008), 0.077 (± 0.027), 0.105 (± 0.005), 0.180 (± 0.035), 0.303 (± 0.016), 0.422 (± 0.017) and 0.523 (± 0.019) mg/L for periods up to 96 h. Twenty organisms were tested at each concentration in 57-L glass aquaria supplied with 4 L/min ozonated water. Mortality observations, water quality data and OPO concentrations were recorded at 6, 12, 24, 48, 60, 72 and 96 h. Death criteria used for fingerlings were similar to those used for larvae. The mean water quality during acclimation and testing was: temperature, 25.2 (± 0.2); salinity, 9.8 (± 0.5) °/oo; pH 7.2 (± 0.2) and dissolved oxygen which ranged from 6.3 to 12.0 mg/L for nominal OPO concentrations of 0.0 to 0.5 mg/L.

Egg, larvae and fingerling studies (Statistical analyses)--Analysis of these data included percentage mortality predictive models for larvae and fingerling, estimation of LC50 values by probit regression (Helwig and Council, 1979) and comparisons of sensitivity between striped bass eggs in fresh and estuarine water. Analysis of variance was used to make comparisons between developmental stages and different salinities.

Atlantic Menhaden--

The acute toxicity of OPO was evaluated for juvenile Atlantic menhaden (*Brevoortia tyrannus*). Fish (8.7 ± 0.7 cm TL; 11.0 ± 2.2 g) were collected from the Patuxent River near Benedict, MD or from Potomac Electric Power Company's Morgantown Generating Station on the Potomac River near Morgantown, MD. Fish were collected using beach seines or cast nets and returned to the laboratory in large aerated tanks. Atlantic menhaden were held at the laboratory, in large 800-L fiberglass tanks supplied with a continuous-flow of 25°C filtered Patuxent River water for two weeks prior to experimentation under a 16 h light: 8 h dark photoperiod. During acclimation, Atlantic menhaden were fed finely ground (grade 000) Purina trout chow. Mortality during the acclimation period was minimal after the first two days. Water quality for the Atlantic menhaden study was the same as that for juvenile blue crabs given above.

Experimental procedures--Eighty Atlantic menhaden were continuously exposed to each OPO concentration of 0.000, 0.079 (± 0.012), 0.120 (± 0.026), 0.209 (± 0.018) and 0.324 (± 0.018) mg/L for 96 h at 4 L/min in 110-L glass aquaria. Mortality was recorded at 12, 24, 48, 72 and 96 h exposure; fish were not fed during the study.

Statistical analyses--Data analyses for juvenile Atlantic menhaden were identical to the procedures performed for the juvenile blue crab data.

White Perch--

Adult white perch (*Morone americana*) were used to 1) evaluate the acute toxicity of OPO; 2) elucidate the physiological basis of acute toxicity and 3) determine the possible recovery potential of fish exposed to sublethal concentrations of OPO. White perch were collected by beach seine or trawl from the Patuxent River near Benedict, MD when ambient temperatures approximated the desired experimental temperature of 15°C. Before experimentation, white perch were acclimated for at least 10 d in 1900- or 3800-L fiberglass holding tanks supplied with a continuous flow of unfiltered Patuxent River water. Fish were fed once daily during acclimation with frozen shrimp or fresh clams and maintained under a 12 h light: 12 h dark photoperiod. The white perch used in these studies had a mean total length, standard length and total wet weight of 164.7 (± 16.6) mm, 135.9 (± 12.7) mm and 54.1 (± 15.3) g, respectively.

Acute studies (Experimental procedures)--Acute toxicity studies were conducted to define dose-mortality relationships for adult white perch exposed to a series of OPO concentrations. Ten fish were exposed to each concentration of 0.000, 0.064 (± 0.007), 0.094 (± 0.019), 0.161 (± 0.011), 0.216 (± 0.015), 0.259 (± 0.016), 0.298 (± 0.013), 0.301 (± 0.016), 0.331 (± 0.014), 0.335 (± 0.011), 0.391 (± 0.014) and 0.424 (± 0.018) mg OPO/L for 96 h at 15°C. Fish were exposed to the various test concentrations in 45-L glass aquaria each supplied with a continuous flow of ≈ 2 L/min of the appropriate test solution. Mean water quality during the acute study was: temperature, 15.0 (± 0.1)°C; salinity, 12.7 (± 0.3) °/oo; pH, 8.0 (± 0.0) and dissolved oxygen which ranged from 11.0 to 17.0 mg/L for nominal OPO concentrations of 0.0 to 0.4 mg/L, respectively. Cessation of opercular movement for 1 min was used as the criterion for death.

Acute studies (Statistical analyses)--Toxicity data were analyzed by percentage mortality modeling techniques described previously along with a standard probit analysis (Helwig and Council, 1979) to calculate LC50 values which are more easily compared with the literature.

Exposure and recovery studies (Experimental procedures)--Sublethal OPO concentrations were selected for the exposure and recovery studies based upon the results of the acute toxicity study. Thirty adult white perch were exposed to each OPO concentration of 0.000, 0.011 (± 0.002), 0.051 (± 0.010), 0.101 (± 0.040) and 0.152 (± 0.045) mg/L for 96 h. At the end of the 96-h exposure period, the flow of ozonated water was stopped and all tanks were purged of ozonated water. Fish were then maintained with a continuous-flow (≈ 2 L/min) of clean unfiltered estuarine water for the next 14 d. Two physiological parameters (blood pH; hematocrit) and one histopathological parameter (gill tissue) were monitored during the 18-d exposure-recovery experiment. These parameters were measured on sets of five fish, randomly selected from each exposure and control tank following 24, 48 and 96 h of exposure and after 4 and 14 d recovery. Fish were individually dip-netted at each sample time from the exposure tanks and quickly wrapped in paper towels to facilitate handling. A scalpel was then used to cut the afferent gill artery under the right operculum. This procedure caused blood to collect in the shallow cavity produced by the incision and was withdrawn using a heparinized Pasteur pipette. Three preheparinized capillary tubes were filled from the pipett and the remaining blood placed in a 3 mL heparinized polyethylene vial. pH was measured immediately using a mico pH electrode (Model MI-410; Microelectrodes Inc., Londonderry, NH). The capillary tubes were centrifuged in a microhematocrit centrifuge for 5 min at 2000 x g and measured using a standard hematocrit reader. The average time from netting to completion of blood collection was approximately 50 s.

After collection of the blood sample, 3 to 4 of the anterior gill arches were removed from the right opercular cavity for histological preparation. Whole gill arches were immediately rinsed in an isosmotic sucrose solution to remove excess blood. For light microscopy, 1 to 2 gill arches were placed in Davidson's fixative for 96 h. After fixation, gills were transferred to 50% ethanol for 24 h and then to 70% ethanol for final dehydration before embedding. Gills were then embedded in paraffin, sectioned sagittally and parasagittally at 6 to 8 μm, stained with hematoxylin and eosin and mounted on glass slides for light microscopy. For scanning electron microscopy (SEM), 1 to 2 gill arches were fixed in a buffered 4% commercial formaldehyde - 1% glutaraldehyde solution (McDowell and Trump, 1976). Additional SEM preparation and photographic work was performed by the Department of Pathology at the University of Maryland's School of Medicine (Baltimore, MD).

Basic water quality during the exposure and recovery periods was: temperature, 15.0 (± 0.2)°C; salinity, 9.0 (± 0.5) ^{o}/oo; pH, 7.9 (± 0.2) and dissolved oxygen which ranged from 8.7 to 11.7 mg/L for nominal OPO concentrations of 0.00 to 0.15 mg/L, respectively.

Exposure and recovery studies (Statistical analyses)--Statistical analyses of blood pH and hematocrit were performed by standard ANOVA procedures followed by a Duncan's multiple range test. An arcsine transformation was performed on hematocrit values before analysis since this parameter is expressed as a percentage; pH data were analyzed directly.

SECTION 4

RESULTS AND DISCUSSION

CHEMISTRY STUDIES

Ozone-Produced Oxidant Decay

The rates of OPO decay in natural, unfiltered Patuxent River estuarine water at 15 and 25°C are shown in Tables 2 and 3, respectively. OPO decay rates at both temperatures followed an approximate first order decay characterized by an initial fast component followed by a much slower component towards the end of decay. Similar observations have been made by other investigators (Blogoslawski et al., 1976 and Marine Research Inc., 1976). The pattern of decay was similar at all concentrations at both temperatures with the exception of the 0.01 mg OPO/L test at 25°C. The rate of decay in this experiment was inexplicably much slower than the rates found in the other seven experiments. The initial rate of decay appeared to be faster at the higher temperature, however, this was not analyzed statistically. This finding is consistent with that of Marine Research Inc. (1976) who found that OPO decay rates were temperature-dependent.

OPO decay in fresh water (Table 4) followed approximate first order decay similar to that observed in estuarine water. However, the rate of decay in freshwater at 17°C relative to that in estuarine water at both 15 and 25°C was faster. The slower oxidant decay in estuarine water relative to fresh water is a result of the more stable oxidizing bromine compounds (e.g. HOBr, OBr^-, bromamines, etc.) found in estuarine water after ozonation or chlorination (Helz, in press). The primary oxidant species in fresh water is O_3 (Kosak-Channing and Helz, 1979). Ozone is a very unstable molecule relative to the oxidizing bromine compounds; therefore, it decays at a much faster rate under similar conditions.

Oxidant Decay and Bromate Formation

Chlorine--

The oxidant decay and concomitant bromate formation for a series of samples chlorinated to an initial concentration of approximately 5 mg/L (71 μM) are shown graphically in Figure 3.

TABLE 2. OZONE-PRODUCED OXIDANT (OPO) DECAY IN NATURAL ESTUARINE WATER AT 15°C (see text for details)*

Time (h)	Nominal Initial OPO Concentration (mg/L)			
	1.00	0.50	0.10	0.01
0.00	100.0	100.0	100.0	100.0
0.25	82.5	66.7	55.2	40.0
0.50	58.3	52.1	38.5	20.0
0.75	48.5	39.6	27.1	10.0
1.00	36.9	32.7	21.9	5.0
2.00	25.0	22.7	13.5	
3.00	19.0	18.3	8.3	
4.00		11.7		
4.50	10.9			
6.50			6.3	
8.00		4.8		
8.50	5.0			
9.50			3.1	
11.00		3.8		
11.50	3.8			
15.50			2.1	
17.00		1.9		
17.50	2.7			
21.50			0.5	
23.0		1.5		
23.5	1.8			

* Values expressed as a percentage of the initial OPO concentration.

TABLE 3. OZONE-PRODUCED OXIDANT (OPO) DECAY IN NATURAL ESTUARINE WATER AT 25°C (see test for detail)*

Time (h)	Nominal Initial OPO Concentration (mg/L)			
	1.00	0.50	0.10	0.01
0.00	100.0	100.0	100.0	100.0
0.25			36.5	
0.33	57.6	37.5		
0.50			25.0	45.5
0.67	47.9	25.4		
1.00	34.6	18.8	17.7	36.4
1.50			15.6	
2.00	21.8	10.4	14.6	27.3
3.00	14.9	6.9	11.5	†
4.00	11.7	5.8	9.4	
6.00	8.6	4.0	8.3	
8.00	5.4	3.1	6.3	
10.00	4.3	2.5		
14.00	3.1	1.7		
18.00	2.2	1.3		
22.00	1.7	1.0		
26.00	1.1			

*Values expressed as a percentage of the initial OPO concentration.

†Accidental loss of sample.

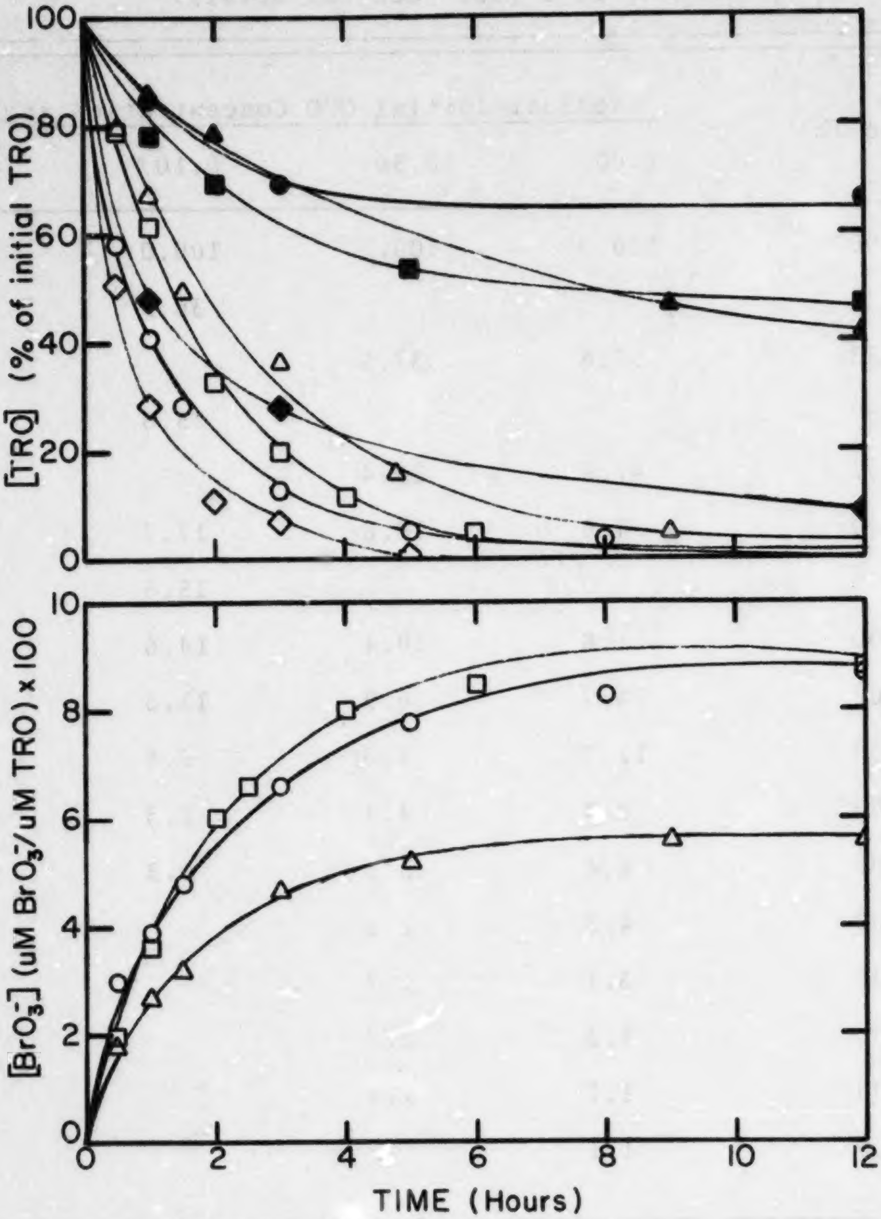

Figure 3. Chlorine decay and bromate formation patterns in light (open) and dark (closed) exposed samples of 5 (○, ●), 15 (□, ■) and 30 °/oo (△, ▲) Instant Ocean and ≈ 5 °/oo Patuxent River water (◇, ◆) following chlorination to an initial total residual oxidant (TRO) concentration of ≈ 5 mg/L (70 μM).

Residual oxidant levels are expressed as a percentage of the initial residual oxidant level to normalize the data since it was impossible to reproduce exactly the same initial concentration for each test series. Bromate concentrations were normalized by calculating the molar ratio of bromate produced to the initial total residual oxidant concentration, measured approximately 30 s following addition of the hypochlorite.

TABLE 4. OZONE-PRODUCED OXIDANT (OPO) DECAY IN FRESH WATER AT 17°C (see text for details)*

Time (h)	Nominal Initial OPO Concentration (mg/L)		
	0.10	0.05	0.01
0.00	100.0	100.0	100.0
0.17			33.3
0.33	59.4	42.0	16.7
0.50			5.6
0.67	40.6	22.0	
1.00	29.7	16.0	
2.00	19.8	6.0	
3.00	8.9	3.0	
4.00	6.9	1.0	
6.00	3.5		
8.00	1.9		
10.00	0.5		

*Values expressed as a percentage of the initial OPO concentration.

In all cases, decay rates were much higher for samples exposed under the sunlamps than for samples left in complete darkness. In artificial sea water, decay rates were inversely related (not proportionally) to salinity for both photolyzed and non-photolyzed samples, although the decay curve for the 30 ^{o}/oo dark samples did overlap the decay rates for the 5 and 15 ^{o}/oo dark samples during the first 6 to 8 h of the decay period. Estuarine water samples (4.0 to 6.5 ^{o}/oo) exhibited greater decay rates than all artificial sea water samples in both the lighted and dark exposure conditions.

Large amounts of bromate formed in the light-exposed lower salinity, artificial sea water samples. No bromate formed in either the photolyzed estuarine water samples or any of the chlorinated samples left in darkness. As shown in Figure 3, there was little difference in bromate formation between the 5 and 15 ^{o}/oo samples during the first 12 h. However, bromate levels in the 5 ^{o}/oo samples continued to increase over the next 12 h while only a marginal amount of additional bromate was formed in the 15 ^{o}/oo sample.

These findings contrast with the experiments of Macalady et al. (1977) who reported sunlight-induced bromate formation levels approximately 3 to 5 times the maximum amount formed in our chlorinated samples (0.88 mg BrO_3^-/L; 6.9 µM). This difference is probably due to the somewhat higher sunlight intensity in southern Florida compared to our sunlamps since Macalady et al. found bromate formation to be directly related to sunlight intensity.

Ozone--

Oxidant decay and bromate formation for 5.0 mg/L (70.5 µM) ozonated samples are shown in Figure 4. They differed from the chlorine experiments in 1) the rate of oxidant decay, 2) the total amount of bromate formed and 3) the requirement of light for bromate formation. Decay rates for all photolyzed, ozonated samples were greater than the decay rates for their respective chlorinated samples, with the difference being most pronounced in 5 ^{o}/oo artificial sea water. No clear pattern was found between the chlorinated or ozonated non-photolyzed samples. Unlike the non-photolyzed, chlorinated samples, however, the decay rates of the dark ozonated samples decreased as the salinity increased.

Bromate formation in photolyzed and non-photolyzed ozone samples is shown in the lower section of Figure 4 and is compared with bromate production in the chlorinated samples in Table 5. In all cases, ozonation induced more bromate formation than chlorination under identical conditions. As can be seen in the lower half of Figure 4, the majority of the total bromate is formed non-photolytically during the actual bubbling, or contacting period at each artificial sea water concentration, while a smaller, additional amount is formed later through photolysis. Bromate concentrations in the

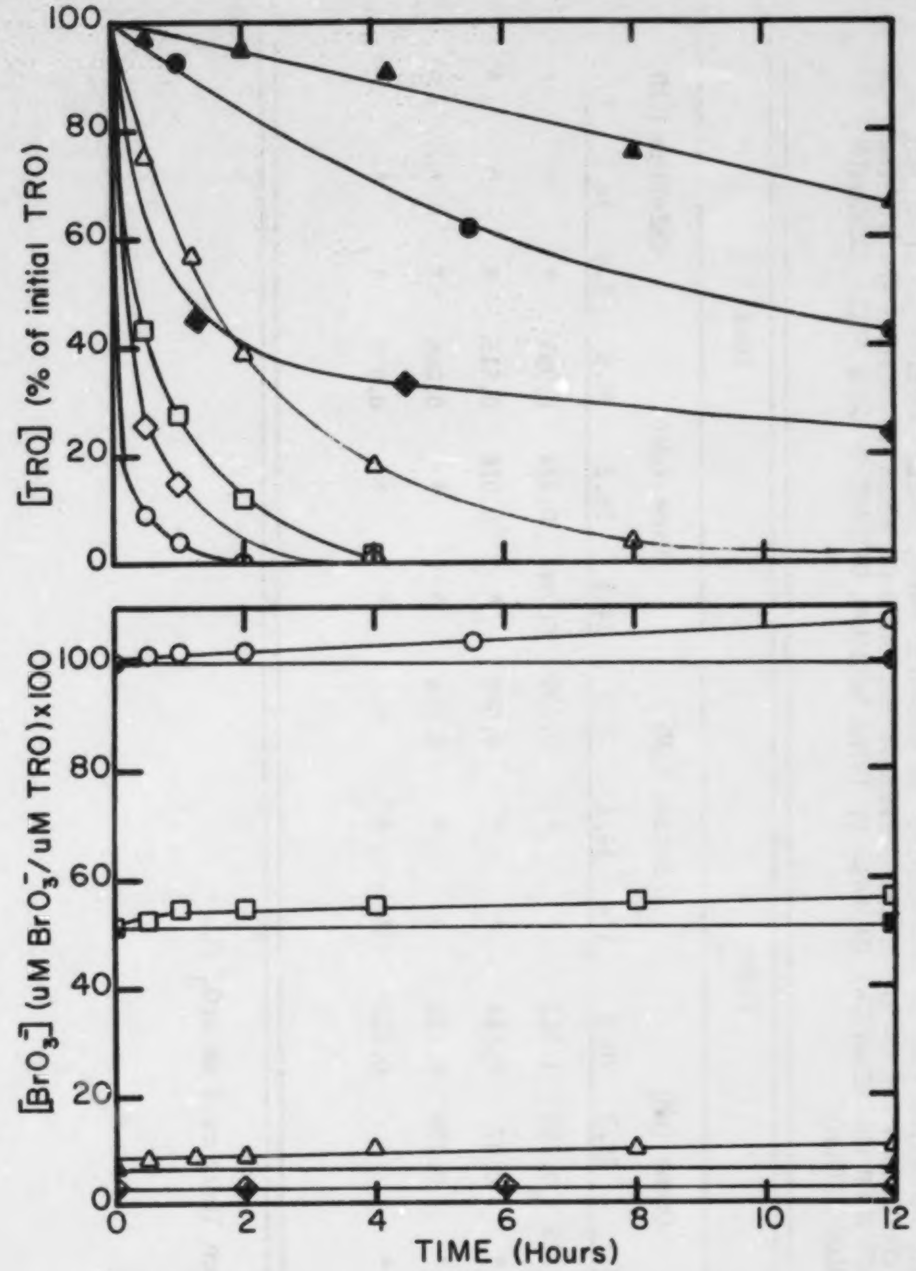

Figure 4. Ozone decay and bromate formation patterns in light (open) and dark (closed) exposed samples of 5 (○, ●), 15 (□, ■) and 30 °/oo (△, ▲) Instant Ocean and ≈ 5 °/oo Patuxent River water (◇, ◆) following ozonation to an initial total residual oxidant (TRO) concentration of ≈ 5 mg/L (70 μM).

TABLE 5. BROMATE FORMATION (µM) IN CHLORINATED AND OZONATED WATER SAMPLES EXPOSED TO LIGHT AND DARK CONDITIONS FOR 24 h. VALUES REPRESENT THE RATIO OF MOLES OF BROMATE (24 h AFTER OZONATION OR CHLORINATION) TO MOLES OF TOTAL RESIDUAL OXIDANT (≈ 30 s AFTER OZONATION OR CHLORINATION)

	Light						Dark					
	Ozone (µM)			Chlorine (µM)			Ozone (µM)			Chlorine (µM)		
Sample	14.1	28.2	70.5	1.4	14.1	70.5	14.1	28.2	70.5	1.4	14.1	70.5
5 ^{o}/oo	0.125	0.259	1.083	*	*	0.099	0.096	0.224	0.997	*	*	*
15 ^{o}/oo	*	0.07	0.584	*	*	0.090	*	0.016	0.515	*	*	*
30 ^{o}/oo	*	0.035	0.116	*	*	0.058	*	*	0.066	*	*	*
River (≈ 5 ^{o}/oo)	*	*	0.038	*	*	*	*	*	0.038	*	*	*

* < the detection limit of 1 µM BrO_3^-/L.

non-photolyzed samples were extremely stable for the entire 24-h exposure period and neither increased nor decreased from the initial level measured immediately after ozonation. If the initial bromate formation concentrations (dark) are subtracted from the total bromate concentrations produced in the light, the difference represents only the additional bromate formed by photolysis during the light exposure period. This light-induced bromate formation in the ozonated samples is very similar in both quantity and formation rate to the light-induced bromate formation in the chlorinated samples.

Both non-photolytic and photolytic bromate formation, in both ozonated and chlorinated solutions, may be attributed to a common free radical mechanism. Kosak-Channing and Helz (1979) have suggested that the chemistry of ozone in sea water is dominated by two competing processes: direct oxidation of Br^- to OBr^- and a hydroxide catalyzed, free radical decay. The rate of the first process (reaction 1) was studied at 0°C by Taube (1942):

$$O_3 + Br^- \rightarrow OBr^- + O_2 \qquad (1)$$

If Taube's rate constant is correct, then reaction 1 is probably faster than the free radical process in sea water at pH 8, and would control the lifetime of O_3. For small residual ozone concentrations (i.e., $\ll Br^-$), the half-life of O_3 in reaction 1 would be about 30 s at 0°C and presumably less at higher temperatures. This could account for the cessation of bromate formation as soon as treatment with ozone gas is stopped.

According to a recent empirical equation obtained by Morooka et al. (1978), the free radical decay process would consume 0.86 μM/s of O_3 in water at pH 8 and 20°C, assuming an O_3 concentration of 5×10^{-4} M, which is approximately the saturation level in equilibrium with a gas phase containing 5 mole% O_3. For an initial O_3 concentration ten-fold lower, the corresponding rate is 0.054 μM/s. If these rates are representative of the free radical decay mechanism in sea water, then this mechanism is slower than ozone decay by reaction 1, but faster than the bromate production process in our experiments ($\leq 10^{-3}$ μM/s). Unfortunately, the effect of dissolved components in sea water on the rate of the free radical decay mechanism can not be predicted quantitatively from existing kinetic data. Hoigne and Bader (1976), however, have shown that some substances, such as HCO_3^-, inhibit the decay process while others accelerate it.

Although a number of different mechanisms have been proposed to explain free radical decay of ozone in water (Peleg, 1976), a common feature of all is a significant role for the hydroxyl radical, $OH^\cdot$. In the classic Weiss (1935) mechanism, hydroxyl is produced as follows:

$$O_3 + OH^- \rightarrow O_2^- + HO_2 \tag{2}$$

$$O_3 + HO_2 \rightarrow 2O_2 + OH^\cdot \tag{3}$$

Zafiriou (1974) considered the fate of $OH^\cdot$ in sea water containing no HOBr or OBr^- and concluded that the dominant reaction would be:

$$OH^\cdot + Br^- \rightarrow Br^\cdot + OH^- \tag{4}$$

The bromine atom would then associate with a halide ion to from Br_2^- or $BrCl^-$ radical ions. The radical ions probably decay by reaction with trace metals or organic matter or by recombination reactions leading to HOBr and OBr^-.

However, as soon as HOBr and OBr^- begin to accumulate, a new reaction, producing the radical, $BrO^\cdot$, will occur:

$$Br_2^- + BrO^- \rightarrow BrO^\cdot + 2Br^- \tag{5}$$

A subsequent series of fast reactions discussed by Buxton and Dainton (1968) leads to bromate:

$$2BrO^\cdot + H_2O \rightarrow BrO^- + BrO_2^- + 2H^+ \tag{6}$$

$$BrO^\cdot + BrO_2^- \rightarrow BrO_2^\cdot + BrO^- \tag{7}$$

$$OH^\cdot + BrO_2^- \rightarrow BrO_2^\cdot + OH^- \tag{8}$$

$$2BrO_2 + H_2O \rightarrow BrO_2^- + BrO_3^- + 2H^+ \tag{9}$$

The production of $BrO^\cdot$ by free radical attack on BrO^- (represented here by reaction 5) is the critical step in this mechanism, since no bromate can be formed if no hypobromite is present.

Since photolyzing chlorinated and ozonated sea water produced similar amounts of bromate, and since bromate is absent from naturally photolyzed sea water, the photolytic process leading to BrO_3^- must also involve HOBr or OBr^-. Amichai and Treinin (1969) and Amichai et al. (1969) studied photolysis of OBr^- and identified two primary processes:.

$$OBr^- \xrightarrow{h\nu} Br^- + O(^3P) \tag{10}$$

$$OBr^- \xrightarrow{h\nu} Br^\cdot + O^- \tag{11}$$

Yields from these two processes were about equal under their conditions. In neutral solutions, O^- hydrolyzes rapidly to form OH$^\cdot$. The radicals, Br$^\cdot$ and OH$^\cdot$, lead to bromate via reactions 4 to 9.

Amichai and Treinin (1969) also investigated the relative rates of O(^{3}P) reactions with a number of compounds in sea water, including O_2, Br^-, Cl^-, NO_3^-, NO_2^- and CO_3^{-2}. For sea water with a typical dissolved oxygen content of about 6.4 mg/L (200 μM), O(^{3}P) would be mainly consumed by:

$$O(^3P) + O_2 \rightarrow O_3 \tag{12}$$

Reactions with Cl^- and Br^- to form OCl^- and OBr^-, respectively, would also be significant, but the estimated rates of other reactions are more than an order of magnitude slower in sea water than reaction 12. The ozone produced by reaction 12 can generate BrO_3^-, as described previously, although the efficiency would be poor because of ozone loss through reaction 1. Thus, essentially the same mechanism can account for non-photolytic bromate production during ozonation and for photolytic production during chlorination or ozonation.

Factors Affecting Bromate Formation--

The lower sections of Figures 3 and 4 clearly show that the rate and magnitude of light-induced bromate levels are inversely related to the sample salinity. To investigate possible sources for this inhibitory action, three substances, including KBr, were examined. Prior to chlorination, NH_3-N, Patuxent River sediment and KBr were each added to 5 °/oo artificial sea water solutions which were then chlorinated to approximately 5.0 mg/L (71 μM) and incubated at 20°C under the sunlamps. The residual oxidant and bromate formation concentrations were measured following a 6-h exposure (Table 6).

Amino-nitrogen--Both NH_3-N and sediment greatly reduced the residual oxidant decay rate while completely inhibiting bromate formation. It is likely that ammonia and organic amines sharply inhibit BrO_3^- by consuming BrO^- faster than reaction 5. Wajon (1980) found that the rate constant for the following reaction at 20°C was 7.5 x 10^7/M/s:

$$HOBr + NH_3^\circ \rightarrow NH_2Br + H_2O \tag{13}$$

Further bromination of NH_2Br to $NHBr_2$ and NBr_3 also occurs rapidly. The rate constant for reaction 5 has essentially the same value as that for reaction 13 (Buxton and Dainton, 1968); therefore, in sea water, where $HOBr \approx OBr^-$, the relative rates of production of BrO$^\cdot$ and NH_2Br by reactions 5 and 13 will simply be equal to the ratio, Br_2^-/NH_3°.

Assuming that the production rate of Br_2^- is on the order of 0.1 to 1 μM/s (consistent with estimates of free

TABLE 6. EFFECTS OF NH_3-N, PATUXENT RIVER SEDIMENT AND KBr ON TOTAL RESIDUAL OXIDANT (TRO) DECAY AND BROMATE FORMATION (μM BrO_3^-/μM TRO) IN 5 °/oo ARTIFICIAL SEA WATER

Sample	Initial Residual Chlorine	Temp	pH	Percentage TRO (after 6 h)	Bromate Formed (after 6 h)
Instant Ocean (5 °/oo)	76.7 μM (5.44 mg/L)	20°C	8.12	2.2	0.080 μM (0.01 mg/L)
NH_3 - 1.5 mg/L added to Instant Ocean (5 °/oo)	75.6 μM (5.36 mg/L)	20°C	8.17	63.8	*
Sediment - 50 mg/L wet weight added to Instant Ocean (5 °/oo)	69.7 μM (4.94 mg/L)	20°C	8.21	24.3	*
Bromide - 50 mg/L added to Instant Ocean (5 °/oo)	79.4 μM (5.63 mg/L)	20°C	8.08	21.8	0.088 μM (0.011 mg/L)

* < 1 μM BrO_3^-/L.

radical decay of ozone), an upper limit on the steady state concentration of Br_2^- can be found in the case where the only decay process is recombination:

$$2Br_2^- \rightarrow Br_3^- + Br^- \qquad k = 1.8 \times 10^9/M/s \qquad (14)$$

The maximum steady state Br_2^- concentration is found to be on the order of 10^{-8} M. If Br_2^- decays by other paths in addition to reaction 14, its concentration will be lower. On the other hand, total ammonia in natural waters is usually in excess of 10^{-6} M, and in sea water free $NH_3°$ will constitute about 10% of the total ammonia. Free amino acids, which total ≈ 10^{-6} M in sea water (Clark et al., 1972), become N-brominated even faster than NH_3 by reactions analogous to reaction 13. Consequently, until the free amino compounds have become fully halogenated and excess OBr^- has formed, little BrO_3^- can form via reactions 5 to 9.

Amino-nitrogen inhibition of BrO_3^- production by the mechanism already described applied to photolysis as well as ozonation. Thus, the absence of BrO_3^- reported in Table 6 when NH_3 was added to Instant Ocean is readily explained, since NH_3 was stoichiometrically in excess of the chlorine dose. The absence of BrO_3^- when sediment was added probably also involves amino-nitrogen inhibition. Sugam and Helz (1977) found Patuxent water near our laboratory to contain 1 to 5 μM total ammonia and 10 to 25 μM organic amino nitrogen. Aminated compounds formed in this manner are much more stable than hypobromous acid and, since they are amperometrically detectable, may explain our observation of a lower residual oxidant decay rate.

Bromide concentration--Bromide also decreased the decay rate but did not significantly inhibit bromate formation. Therefore, additional tests were run with various concentrations of Br^- in Instant Ocean solutions (Table 7). Three concentrations of artificial sea water (5, 15 and 30 °/oo) were treated with concentrated hypochlorite to yield initial residuals of approximately 5 mg/L (71 μM). Three Br^- solutions were made by adding KBr to 5 °/oo Instant Ocean (9.6 mg/L as Br^-) to yield total bromide concentrations of 20.8, 43.2, and 76.8 mg/L (as Br^-). The Br^- solutions were also chlorinated to approximately 5 mg/L. All samples were placed under sunlamps immediately following chlorination. After a 6-h exposure each sample was analyzed for total residual oxidant and bromate concentrations. As shown in Table 7, there was little difference in the residual oxidant or bromate production levels between the 5 and 15 °/oo artificial sea water solutions. There was, however, a significant increase in the residual oxidant level in the 30 °/oo solution with a corresponding decrease in bromate formation. The three solutions with elevated bromide concen-

TABLE 7. BROMATE (µM BrO_3^-/µM TRO) AND TOTAL RESIDUAL OXIDANT (TRO) CONCENTRATIONS IN THREE ARTIFICIAL SEA WATER CONCENTRATIONS AND THREE KBr CONCENTRATIONS (IN 5 °/oo ARTIFICIAL SEA WATER) 6 h AFTER CHLORINATION TO AN INITIAL RESIDUAL OF = 5 mg/L (70 µM).

Sample	Total Bromide	Time (h)	Percentage TRO	Bromate Formed
Instant Ocean 5 °/oo	120.1 µM (9.6 mg/L)	6	4.5	0.081 µM (0.010 mg/L)
Instant Ocean 15 °/oo	360.4 µM (28.8 mg/L)	6	5.1	0.086 µM (0.011 mg/L)
Instant Ocean 30 °/oo	720.8 µM (57.6 mg/L)	6	11.5	0.055 µM (0.007 mg/L)
11.2 mg/L Br^-* in 5 °/oo Instant Ocean	260.3 µM (20.8 mg/L)	6	12.3	0.144 µM (0.018 mg/L)
33.6 mg/L Br^-* in 5 °/oo Instant Ocean	540.6 µM (43.2 mg/l)	6	15.4	0.140 µM (0.018 mg/L)
67.2 mg/L Br^-* in 5 °/oo Instant Ocean	961.1 µM (76.8 mg/L)	6	26.1	0.097 µM (0.012 mg/L)

* Added as KBr.

trations showed exactly the same pattern for both residual oxidant and bromate responses, but the magnitude of the responses was altered considerably. The percentage of residual oxidant remaining in the solutions with an elevated bromide level was 2.5 to 3.0 times that found in the regular artificial sea water samples and almost twice as much bromate was formed in the samples having the higher bromide concentrations. Blogoslawski et al. (1976) also found the bromide concentration to be related to the residual oxidant concentration following ozonation of natural sea water, and that the residual oxidant was produced at a substantially lower yield than one would expect stoichiometrically. They attributed the loss to formation of bromine gas or other non-reactive bromide species or both; however, our results demonstrate that a portion of Blogoslawski et al.'s (1976) "lost bromine" may have occurred through the production of bromate during the ozone contacting period.

The bromate production mechanism proposed in this report accounts for a number of our observations, but fails to account for others. For example, the effect of Br^- on photolytic BrO_3^- yields is unexplained (Table 7). Almost certainly, the present mechanism is too simple and additional reactions contribute to bromate production. Direct reactions between ozone and various oxybromine species could prove to be kinetically more important than some of the steps suggested here. Furthermore, the $HOBr$-OBr^- equilibrium is strongly dependent on pH. Most of the available kinetic studies have been conducted in a pH range $\geq$ 8 and have primarily focused on the reactions of the hypobromite ion, OBr^-. In many estuarine areas, the pH is usually between 7 and 8 and would thus cause a dramatic increase in the concentration of HOBr. Almost no kinetic data are available with which to evaluate the chemistry of hypobromous acid (HOBr) in estuarine or marine waters. Additional information is needed in this area if the reactions of chlorine and ozone are to be completely understood. In addition, application of laboratory studies conducted in clean sea water may be of limited usefulness in predicting the speciation of residual oxidants and their resulting end products due to the complex and highly variable constituents of most natural waters. It is suggested that more comprehensive studies be conducted on a site specific basis to determine actual residual oxidant composition and that more effort be directed toward identifying other non-amperometrically detectable end products and their potential toxicity to environmentally important species.

TOXICITY STUDIES

Bromate

Striped Bass--

Striped bass (*Morone saxatilis*) eggs (12 h post-fertilization) exposed to bromate concentrations of 0, 10, 20, 50, 75, 100, 200, 300 and 500 mg/L (as BrO_3^-) for a maximum of 36 h exhibited hatch percentages of 95%, 90%, 80%, 85%, 88%, 73%, 83%, 58% and 58%, respectively. A progressively longer delay in hatching time was observed as bromate concentrations increased above 100 mg/L with the greatest delay (≈ 10 h) occurring at 500 mg/L. The hatch-time-temperature relationship for striped bass eggs has been described by Rogers et al. (1977) as:

$$T = 285.5\ e^{-0.0934(t)}$$

where T = time in h from fertilization to hatch and t = water temperature in °C. This relationship gives an expected hatch time ≈ 40 h for eggs raised at 20°C, which closely agrees with the ≈ 38 h from fertilization to hatch observed in our control. In bromate concentrations where delayed hatching was observed ($BrO_3^- \geq 100$ mg/L), all eggs which had not hatched within 10 h of the controls died without hatching.

Mortality data for the egg-prolarval study are listed in Table 8 and graphically depicted in Figure 5. The response surface was statistically predictable at the 0.05 level. Confidence limits for the 24-h exposure data (Table 8) were exceptionally large since the LC50 value was derived by extrapolation beyond the range of concentrations tested. Prolarvae were much more sensitive to bromate than eggs as indicated by the large decrease in LC50 values following hatch. This increased sensitivity is probably due to the absence of a chorion, which acts as a protective covering for the egg during the early stages of development (Middaugh et al., 1978).

Newly hatched prolarvae in concentrations above 75 mg/L (i.e., 100 to 500 mg/L) exhibited progressively greater larval deformation with increasing bromate concentration (qualitative observation). Deformed larvae appeared to be smaller than control larvae and most had various degrees of spinal curvature, while a few in the highest concentrations had enlarged forebrain areas (pugheadedness). These types of physical abnormalities have also been observed for striped bass eggs exposed to severe thermal shock (Koo and Johnston, 1978), and for striped bass and blueback herring (*Alosa aestivalis*) eggs and larvae exposed to total residual chlorine (Morgan and Prince, 1977 and 1978).

The 10-d experiment with striped bass larvae, which were initially 4 d old (i.e., 4 d post-hatch), was conducted to determine if older larvae were more sensitive to bromate than young prolarvae. The results of this experiment are summarized in Table 9 and shown graphically in Figure 6.

TABLE 8. LC50 VALUES AND 95% CONFIDENCE LIMITS FOR STRIPED BASS EGGS-PROLARVAE EXPOSED TO BROMATE UP TO 96 h.

Exposure Time (h)	LC50 (mg/L as BrO_3^-)	95% Confidence Limits
24	697.4	313.01 - 5,974.94
Hatch (26-36 h)		
36	103.2	74.45 - 139.18
58	42.9	29.95 - 56.91
72	39.8	27.51 - 51.44
96	30.8	20.48 - 40.21

TABLE 9. LC50 VALUES AND 95% CONFIDENCE LIMITS FOR 4-d OLD STRIPED BASS LARVAE EXPOSED TO BROMATE UP TO 10 d.

Time (d)	LC50 (mg/L as BrO_3^-)	95% Confidence Limits
1	-	- -
2	605.0	507.0 - 1007.3
3	491.7	438.9 - 621.4
4	403.5	350.3 - 480.4
5	313.4	272.8 - 366.4
6	230.3	199.9 - 271.0
7	197.2	172.3 - 229.1
8	177.4	147.5 - 210.0
9	148.3	114.2 - 185.0
10	92.6	* - *

* Limit undefined by computer calculations.

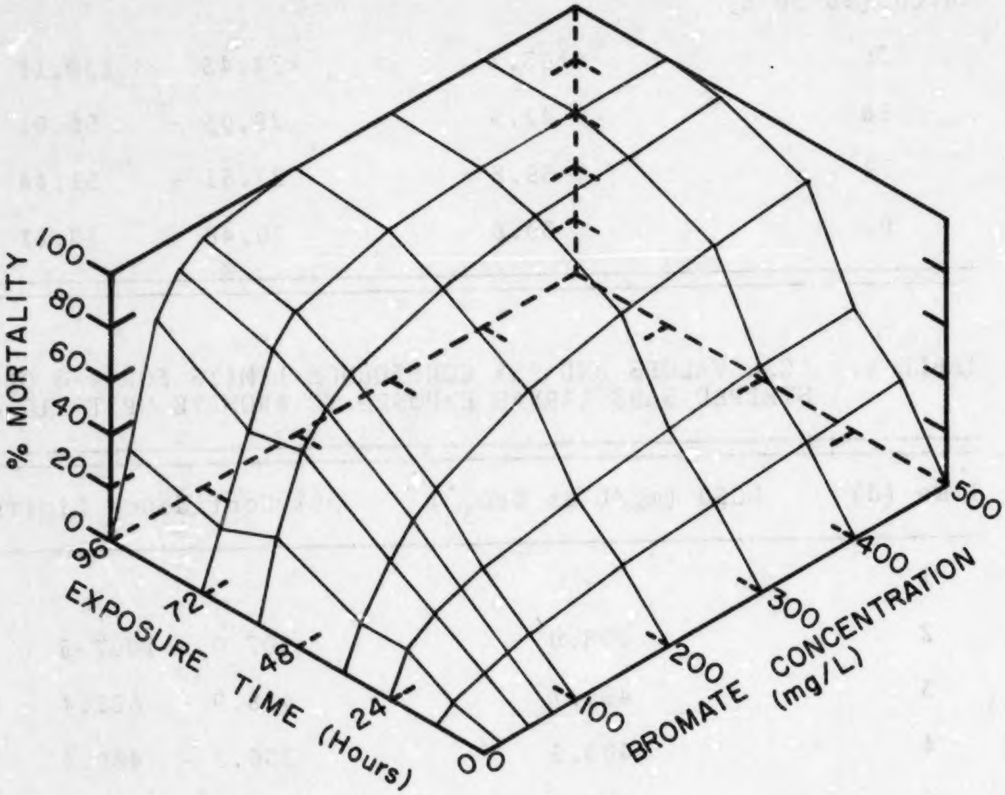

Figure 5. Predicted percentage mortality response surface for the egg-prolarvae stages of the striped bass continuously exposed to bromate up to 96 h.

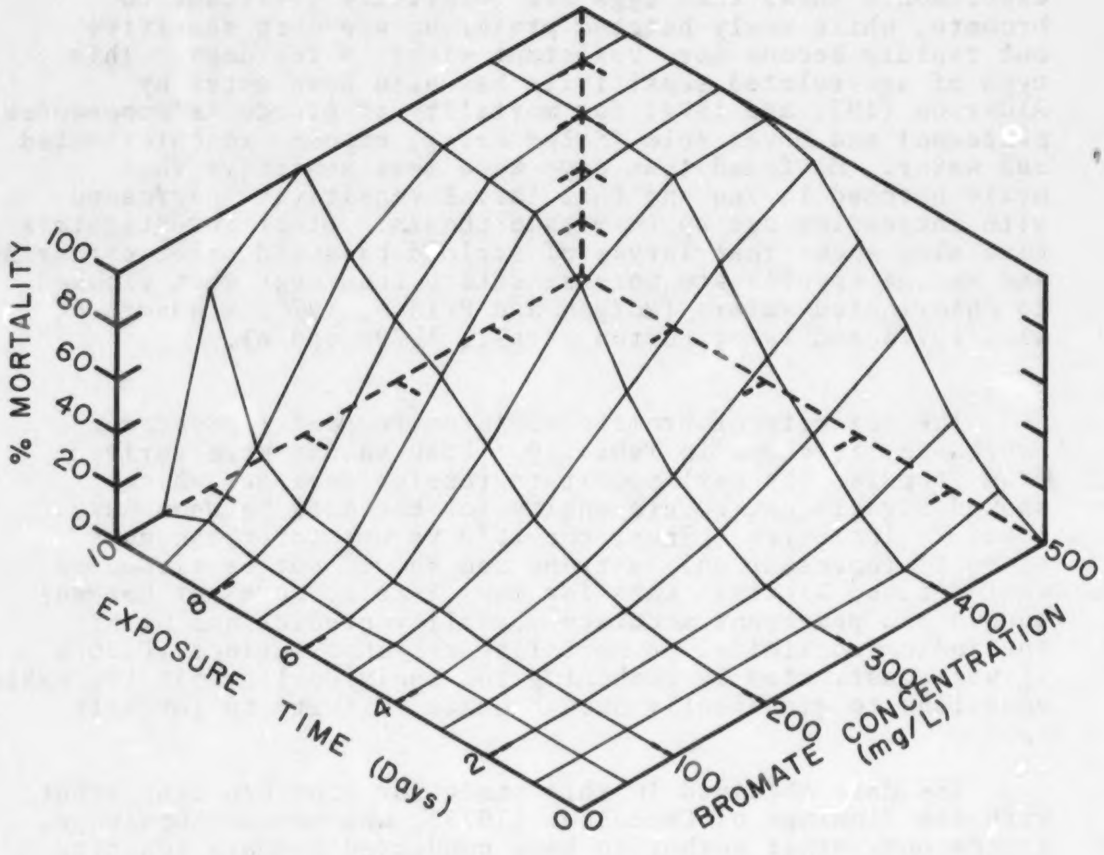

Figure 6. **Predicted percentage mortality response surface for striped bass larvae exposed to bromate up to 10 d.**

The combined chi-square analysis for the response surface in Figure 6 showed that the response surface accurately represents the data at the 99% confidence level. The 1-d mortality data in Figure 6 are the actual percentage mortality since there was only minimal mortality (10%) at the highest concentration and thus not enough data to be analyzed statistically.

A comparison between the egg-prolarvae and larvae experiments shows that eggs are relatively resistant to bromate, while newly hatched prolarvae are more sensitive, but rapidly become more resistant within a few days. This type of age-related sensitivity has also been noted by Alderson (1972 and 1974) for mortality of plaice (*Pleuronectes platessa*) and Dover sole (*Solea solea*) exposed to chlorinated sea water. He found that eggs were less sensitive than newly hatched larvae and that larval sensitivity decreased with increasing age up to metamorphosis. Other investigators have also shown that larvae of striped bass and other estuarine and marine species are more sensitive than eggs when exposed to chlorinated waters (Morgan and Prince, 1977; Middaugh et al., 1977a and 1978; Burton et al., 1979a and b).

Spot--

The toxicity of bromate to juvenile spot (*Leiostomus xanthurus*) is shown in Table 10. LC50 values were derived from stepwise (by day) probit regression analyses which showed significant heterogeneity for the data between days 1 and 7, inclusive. Thus, the LC50 values for these days (1 to 7) represent only a trend and should not be viewed as well defined limits. Data for days 8 to 10 were not heterogenous and represent accurate mortality predictions within the indicated limits. A mortality response surface (Figure 7) was constructed by combining the individual probit regression equations to graphically show bromate toxicity to juvenile spot.

The data obtained in this study for spot are consistent with the findings of Crecelius (1979), who to our knowledge, is the only other author to have conducted bromate toxicity experiments involving marine or estuarine fish. Crecelius (1979) found a 96-h LC50 of 512 mg/L (as BrO_3^-) for the toxicity of bromate to juvenile chum salmon (*Oncorhynchus keta*). This value is the same order of magnitude as that derived from our experiments with juvenile spot, although a quantitative comparison cannot be made due to the heterogeneous nature of our data.

A comparison of bromate toxicity between striped bass and spot shows that newly hatched striped bass prolarvae are most sensitive to bromate, followed by older larvae, eggs and juvenile spot. In contrast, the early developmental stages of some molluscan larvae appear to be an order of magnitude more sensitive than other invertebrates, ichthyoplankton and juvenile and adult fish (Crecelius, 1979; Stewart et al., 1979). Furthermore, Crecelius (1979) and Macalady et al. (1977) have shown, in the laboratory, that bromate can be formed in chlorinated or ozonated seawater at concentrations that could have a deleterious effect on American oyster larvae (Stewart et al., 1979). Thus, bromate concentrations produced from the chlorination or ozonation of natural estuarine or marine water could be great enough to affect some molluscan larvae but would probably have little or no acute effects on other invertebrates, ichthyoplankton and juvenile and adult fish. Sublethal effects of bromate have never been investigated and may be significant for extended exposure periods. We suggest that studies be conducted in the field to determine the amount of bromate that could possibly occur in chlorinated or ozonated estuarine and marine discharges. Additional toxicity studies on the early developmental stages of various molluscs are also needed to verify the potential detrimental effects observed by Stewart et al. (1979).

TABLE 10. LC50 VALUES AND 95% CONFIDENCE LIMITS FOR JUVENILE SPOT EXPOSED TO BROMATE UP TO 10 d.

Time (d)	LC50 (mg/L as BrO_3^-)	95% Confidence Limits
1	698.0†	492.9 - 1946.2
2	517.6†	368.1 - *
3	468.2†	267.6 - 765.3
4	427.4†	322.4 - 685.4
5	415.2†	322.6 - 604.7
6	398.9†	304.1 - 590.3
7	378.9†	293.6 - 527.7
8	337.2	295.4 - 387.1
9	295.8	258.4 - 337.4
10	278.6	243.9 - 316.9

* Limit undefined by computer calculations.
† Significant heterogeneity.

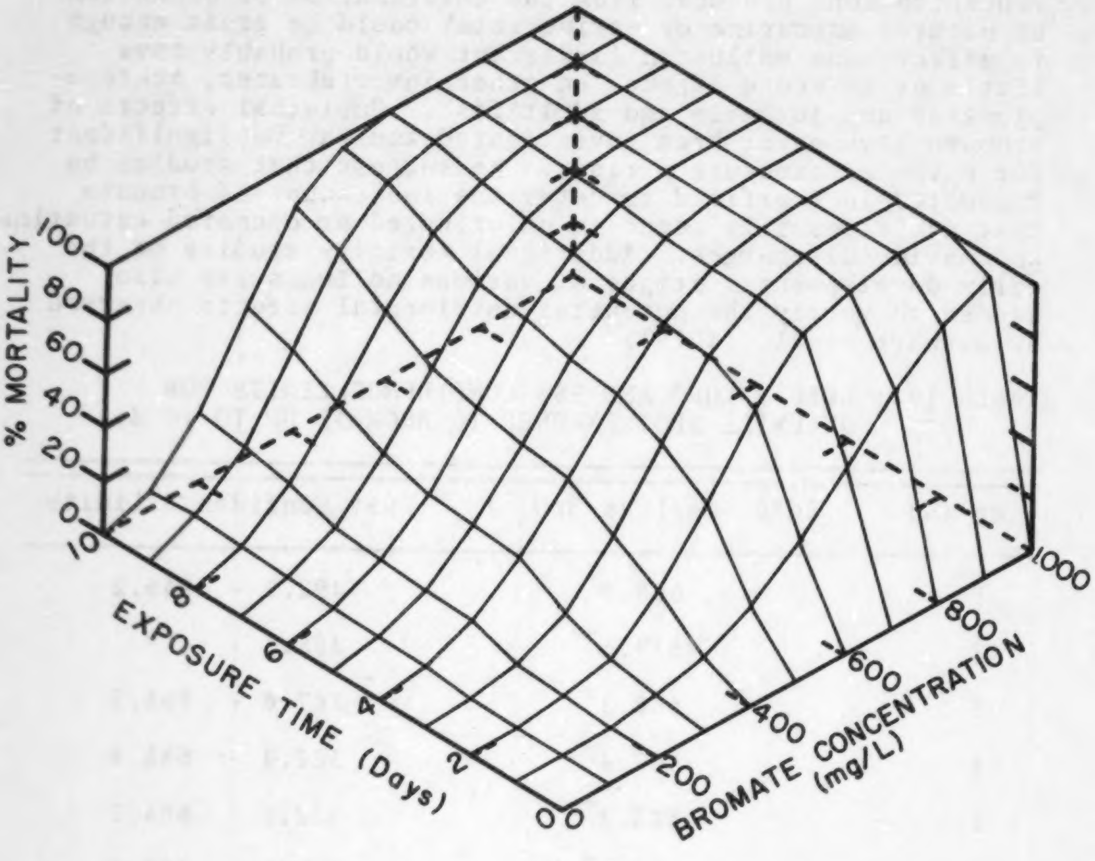

Figure 7. Predicted percentage mortality response surface for juvenile spot exposed to bromate up to 10 d.

Ozone-Produced Oxidants

American Oyster--

Larvae--The final predictive equations for the toxicity of OPO to straight-hinge and setting pediveliger larval stages of the American oyster (*Crassostrea virginica*) are shown in Table 11. The predictive equations for CPO-exposed larvae taken from Roosenburg et al. (1980) are also included in Table 11. Response surfaces for the straight-hinge and setting pediveliger stages are presented in Figures 8 and 9, respectively.

The single most important factor in predicting the mortality of both larval stages exposed to each oxidant was found to be dose (i.e., concentration x time). This suggests that the mode of action is similar for both OPO and CPO. Setting pediveliger larvae, however, were significantly more resistant to the oxidants than straight-hinge larvae (Table 12; Comparison I & II). CPO was found to be significantly more toxic than OPO to both developmental stages (Table 12; Comparison III & IV). This relationship can also be seen by comparing LC50 values from our work with those of Roosenburg et al. (1980). They reported 48-, 72- and 96-h LC50's of 0.30, 0.08 and 0.06 mg CPO/L, respectively, for straight-hinge larvae, while the 48-, 72- and 96-h LC50's in our studies were 0.36, 0.19 and 0.12 mg OPO/L, respectively. LC50 values were not computed for setting pediveliger larvae since the maximum mortality never reached 50%; extrapolation of an arcsine or probit model would predict an LC50 much higher than the true value. The highest mortality for pediveliger larvae exposed to the maximum doses of OPO and CPO was 29.0 and 43.5%, respectively.

The results obtained in this study are the only quantitative data we have seen in the literature which quantify the toxicity of OPO to the larval stages of the American oyster. DeManche et al. (1975) qualitatively described the effectiveness of using various techniques to remove the residual toxicity of ozonized sea water to the Pacific oyster larvae (*C. gigas*) but did not measure the residual oxidant levels. MacLean et al. (1973) studied the effects of ozone-treated sea water on the spawned, fertilized, meiotic and cleaving eggs of the American oyster. Although they did not measure residual oxidant concentrations during the experiment, they estimated that the residual concentration may have been ≈ 0.18 mg OPO/L at the start of the experiment. It is, therefore, not surprising that they found a severe impact on these extremely sensitive developmental stages. Stewart et al. (1979) conducted 48-h static bioassays on fertilized eggs of the American oyster using three by-products which can be formed following ozonation or chlorination.

TABLE 11. PREDICTIVE MODELS FOR PERCENTAGE MORTALITY OF AMERICAN OYSTER STRAIGHT-HINGE AND PEDIVELIGER LARVAE EXPOSED TO OZONE-PRODUCED OXIDANTS (OPO) AND CHLORINE-PRODUCED OXIDANTS (CPO)* UP TO 96 h.

Developmental Stage	Oxidant	Modeling Equation	r^2
Straight-hinge larvae	OPO	$Y = 0.1247 + 0.186D + 0.004DT$	0.93
Straight-hinge larvae*	CPO	$Y = 0.2385 + 0.1090D - 0.2370DC$	0.87
Pediveliger larvae	OPO	$Y = -0.0612 + 0.0086D + 0.0831\ (\ln T)$	0.41
Pediveliger larvae*	CPO	$Y = 0.1989 + 0.6609C + 0.0344D - 0.0009D^2$	0.70

* From Roosenburg et al. (1980).

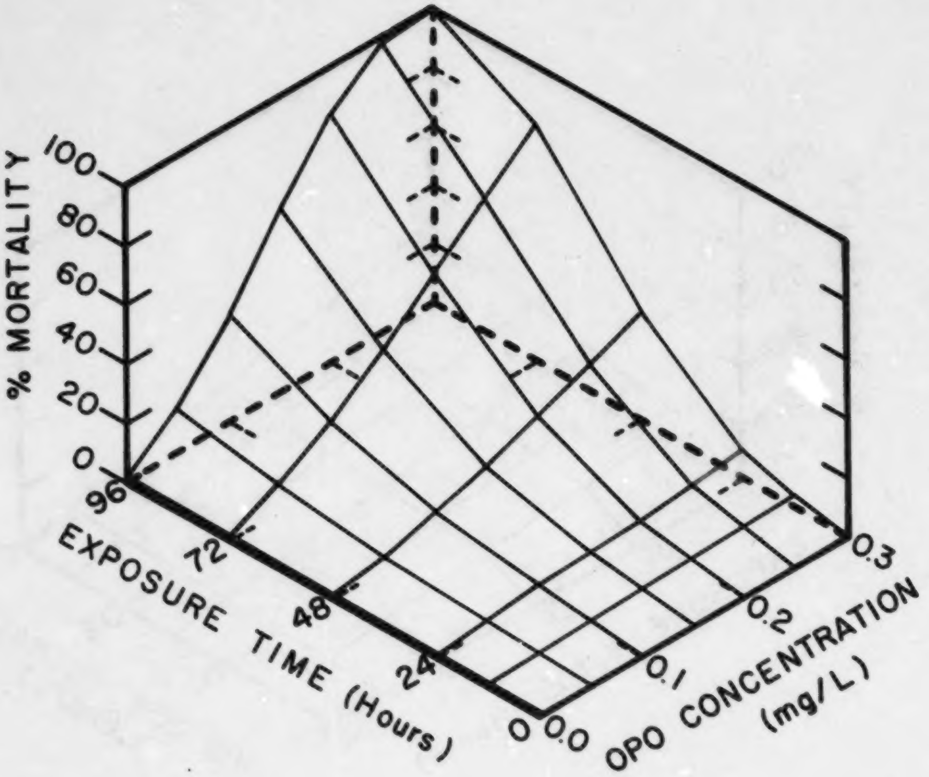

Figure 8. Predicted percentage mortality response surface for American oyster straight-hinge larvae exposed to ozone-produced oxidants (OPO) up to 96 h.

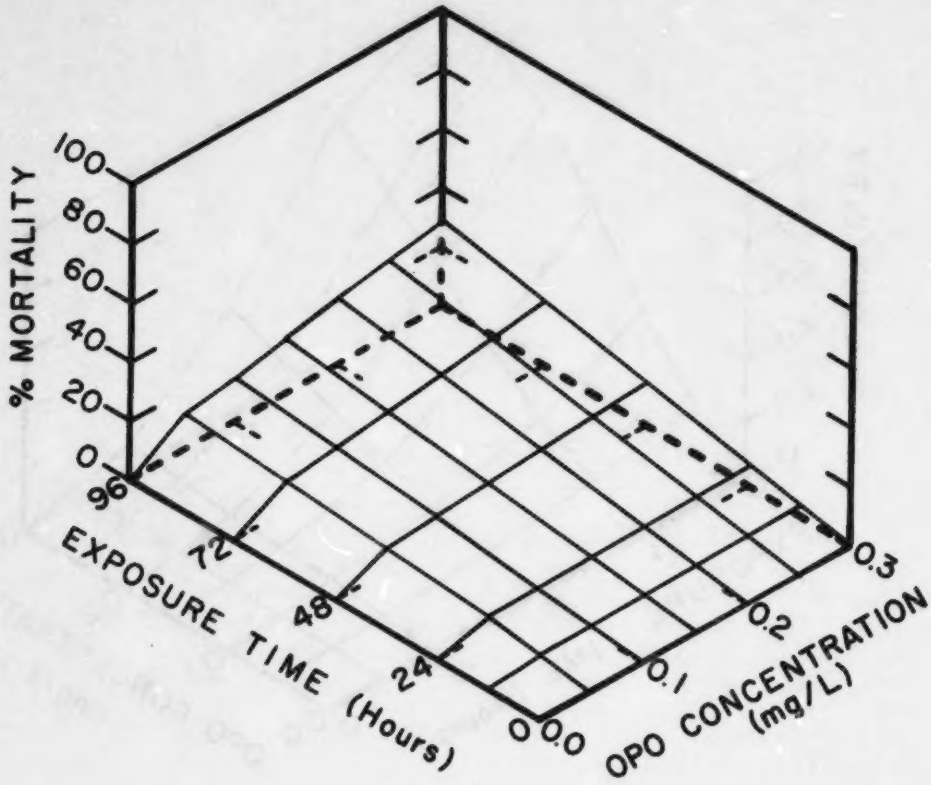

Figure 9. Predicted percentage mortality response surface for American oyster setting pediveliger larvae exposed to ozone-produced oxidants (OPO) up to 96 h.

TABLE 12. ANOVA STATISTICS COMPARING PERCENTAGE MORTALITY FROM THE PREDICTIVE MODELS OF BOTH AMERICAN OYSTER LIFE STAGES (STRAIGHT-HINGE; PEDIVELIGER) AND OXIDANTS (OPO, CPO).

	Comparison	Source	df[*]	MS[†]	F[§]	P[ψ]
I	OPO: Straight-hinge--pediveliger	life stage	8	0.203	13.68	0.001
		error	183	0.015		
II	CPO: Straight-hinge--pediveliger[#]	life stage	8	0.297	20.01	0.001
		error	183	0.015		
III	Straight-hinge: OPO-CPO[#]	oxidant	8	0.087	5.84	0.001
		error	183	0.015		
IV	Pediveliger: OPO-CPO[#]	oxidant	8	0.188	12.69	0.001
		error	183	0.015		

[*] Degrees of freedom.
[†] Mean square.
[§] F-statistic.
[ψ] Significance level.
[#] From Roosenburg et al. (1980).

They found a marked decrease in survival with chloroform, bromoform and bromate concentrations as low as 0.05 to 0.10 mg/L. Unfortunately, we did not measure these during the course of this study. However, our bromate chemistry studies indicate that bromate formation does not occur to any great extent in natural waters due to the presence of aminated and complex organic compounds.

More work has been conducted on the toxicity of CPO to larval American oysters (Roosenburg et al., 1980). Although the responses of oyster larvae exposed to OPO and CPO were statistically different (Table 12), the response surface configurations appeared quite similar for the two oxidants (see Roosenburg et al., 1980). It is possible, when fitting a polynomial response surface model with a sparse set of independent variables, for the predictive model to reflect anomalies in the data that could have resulted only from random deviation from the mechanism that controls mortality. When this occurs, the model "overfits" the data. Consequently, a high goodness-of-fit for each lifestage-oxidant model and the relatively small number of independent variables in each experiment may have produced a significant difference due to "overfitting" of the response surfaces.

Our results also agree with the data from Waugh (1964), who found that short exposures (3 to 20 min) to high CPO concentrations had negligible effect on early *Ostrea edulis* larvae at concentrations up to 10 mg/L. Roberts and Gleeson (1978), however, showed that the 48-h LC50 for American oyster larvae during the trochophore stage was ≈ 0.02 to 0.03 mg CPO/L. The trochophore stage, therefore, is an order of magnitude more sensitive to both OPO and CPO than the early straight-hinge stage, while pediveliger larvae are even less sensitive than the straight-hinge larvae. These data clearly show that the sensitivity of larval oysters to CPO and OPO is greatest immediately following fertilization and decreases with larval age up to the pediveliger stage of larval development. This increase in resistance with age is probably due to the progressive development of a thicker and more complete shell.

Adults--The results of the shell deposition experiment are shown in Table 13. Shell deposition values (normalized) at 15 and 25°C were not significantly different ($p > 0.05$) at any of the OPO exposure concentrations. As can be seen from the table, the same response pattern occurred at both 15 and 25°C. Shell deposition for control oysters was significantly greater than all exposure concentrations.

Fecal accumulation (production of feces and pseudofeces) for oysters exposed to the same OPO concentrations at 15 and 25°C is summarized in Table 14 and shown graphically in

TABLE 13. MULTIPLE RANGE STATISTICS ($p < 0.05$) OF SHELL DEPOSITION* CHANGES IN ADULT AMERICAN OYSTERS EXPOSED TO OZONE-PRODUCED OXIDANTS (OPO) AT 15 and 25°C (see text for details)

Temperature	Parameter	Nominal OPO Concentration (mg/L)						
		0.00	0.01	0.05	0.10	0.20	0.30	0.40
15°C	Mean (mm)	0.93	0.38	0.26	0.20	0.08	0.11	0.04
	± SD	0.22	0.14	0.06	0.07	0.04	0.04	0.03
	% of control	-	41	28	22	9	12	4
25°C	Mean (mm)	0.96	0.22	0.30	0.06	0.11	0.04	0.06
	± SD	0.26	0.09	0.11	0.02	0.07	0.02	0.06
	% of control	-	23	31	6	11	4	6

* Normalized for a 96-h exposure.

TABLE 14. FECAL ACCUMULATION* RESULTS FOR ADULT AMERICAN OYSTERS EXPOSED TO OZONE-PRODUCED OXIDANTS (OPO) AT 15 AND 25°C (see text for details)

Temperature	Parameter	Nominal OPO Concentrations (mg/L)						
		0.00	0.01	0.05	0.10	0.20	0.30	0.50
15°C	mg/g wet wt.*	26.0	27.6	19.2	13.6	11.2	12.0	10.8
	% of control	-	106	73	53	42	45	41
25°C	mg/g wet wt.*	77.6	68.2	31.8	20.6	10.9	12.2	9.2
	% of control	-	88	41	26	14	16	12

* Expressed as: mg fecal matter/g wet oyster weight/96 h.

Figure 10. Fecal accumulation at both temperatures was greatest in the control group and decreased as the concentration increased for OPO-exposed groups. The relationship between reduced fecal matter accumulation and OPO concentration, following regression analysis, was found to be:

$$15^\circ\text{C} \qquad F = 8.078 + 1.116 \div (\text{conc} + 0.562)$$

$$25^\circ\text{C} \qquad F = 10.562 + 1.078 \div (\text{conc} + 0.0143)$$

where

F = fecal accumulation (mg/g wet oyster weight/96 h)

conc = exposure concentration (mg/L)

A paired t-test showed no significant difference ($p < 0.05$) between the normalized fecal accumulation values at 15 and 25°C for OPO concentrations greater than ≈ 0.10 mg/L. However, fecal accumulation for 15°C-acclimated oysters was significantly lower than for 25°C-acclimated oysters at concentrations < 0.10 mg OPO/L. Approximate EC50 values for the 15 and 25°C fecal accumulation experiments were 0.13 and 0.02 mg OPO/L, respectively.

Once larvae have set and metamorphosed, the adult form becomes progressively more tolerant with age to residual oxidants. This study and others (Rhoderick et al., 1977; Liden et al., 1980) have shown that adult oysters, at least 1-year old, are capable of surviving 1- to 2-week exposures to high OPO and CPO concentrations. This study also shows that at residual oxidant concentrations greater than ≈ 0.20 mg OPO/L, oysters remained closed almost constantly and did not produce fecal matter. Death would thus result indirectly from starvation or asphyxiation if the exposure time exceeded an oyster's ability to sustain itself through anaerobic metabolism. Below 0.10 mg OPO/L oysters began to produce more fecal matter as concentrations became smaller (Figure 10) and remained open for longer periods (qualitative observation). These fecal accumulation data agree with those of Scott and Middaugh (1978) who found a significant reduction in fecal production in oysters exposed to 0.12 mg CPO/L or higher concentrations. Shell deposition, however, was affected at even lower concentrations (0.01 mg OPO/L). Although the exact EC50 was not calculated, our data indicate that it lies below 0.01 mg OPO/L. Similarly, Sheridan (in press) has found that the growth of American oyster spat is unaffected at ≈ 0.003 mg CPO/L and significantly reduced at ≈ 0.01 mg CPO/L.

Thus, the growth of oysters continuously exposed to OPO

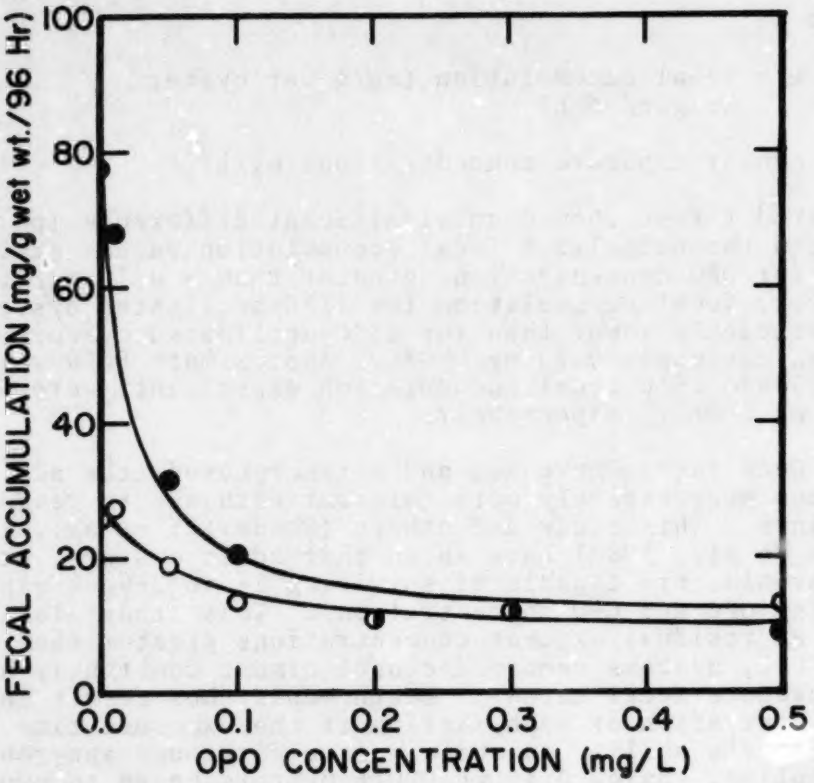

Figure 10. Fecal accumulation for 15 (○) and 25°C (●) acclimated adult American oysters exposed to ozone-produced oxidants (OPO) after standardizing for a 96-h exposure period.

or CPO concentrations of 0.01 mg/L or more for extended periods may be inhibited. Concentrations $\geq$ 0.05 mg/L would result in almost no growth, poor oyster quality and, therefore, the eventual decline of the exposed population. These assumptions, however, are based on a continuous exposure with water quality similar to that in this study; intermittent or periodic exposure would probably result in a markedly lower impact.

Blue Crab--

The final predictive equation for percentage mortality of juvenile blue crabs (*Callinectes sapidus*) at 25°C exposed to OPO for periods up to 144 h was:

$$Y = 0.9551 + 2.4964C + 0.2272(\log T) \qquad r^2 = 0.84$$

Percentage mortality as a function of OPO concentration and exposure time is shown graphically in Figure 11.

The equation for juvenile blue crabs indicates that OPO concentration (C) and the natural logarithm of time (logT) are the only significant ($p < 0.10$) independent variables which predict the mortality of this species during continuous exposure to CPO. This is clearly shown in Figure 11 where mortality increases logrithmically parallel to the exposure time axis and in the flattened sigmoid fashion characteristic of the arcsine function along the concentration axis. Both terms made highly significant ($p < 0.0001$) contributions to the model. However, a comparison of partial F-values showed concentration to be the most important of the two variables.

Table 15 lists the LC50 values for juvenile blue crabs calculated from both the modeling equation and repeated probit regression. In all cases except one (6-h LC50), the LC50's computed from the modelling equation fell well within the 95% confidence limits derived from standard probit techniques.

Juvenile blue crabs were much more resistent to OPO relative to the other species in this study. Laird and Roberts (1980) reported 48- and 96-h LC50's of 0.42 and 0.32 mg/L, respectively, for juvenile blue crabs exposed to CPO. These values are similar to the 48- and 96-h LC50's of 0.35 (0.276 to 0.449) and 0.26 (0.160 to 0.308) mg OPO/L, respectively, found in this study. Burton and Margrey (1978) also found comparable LC50's for juvenile blue crabs exposed to bromine chloride-produced oxidants. It appears that adult blue crabs may be more resistant than juveniles exposed to oxidants in estuarine water. Vreenegoor et al. (1977) found 48-, 96- and 144-h LC50's for adult blue crabs tested at 25°C to be 0.75, 0.70 and 0.63 mg CPO/L, respectively. These values are considerably higher than those found for

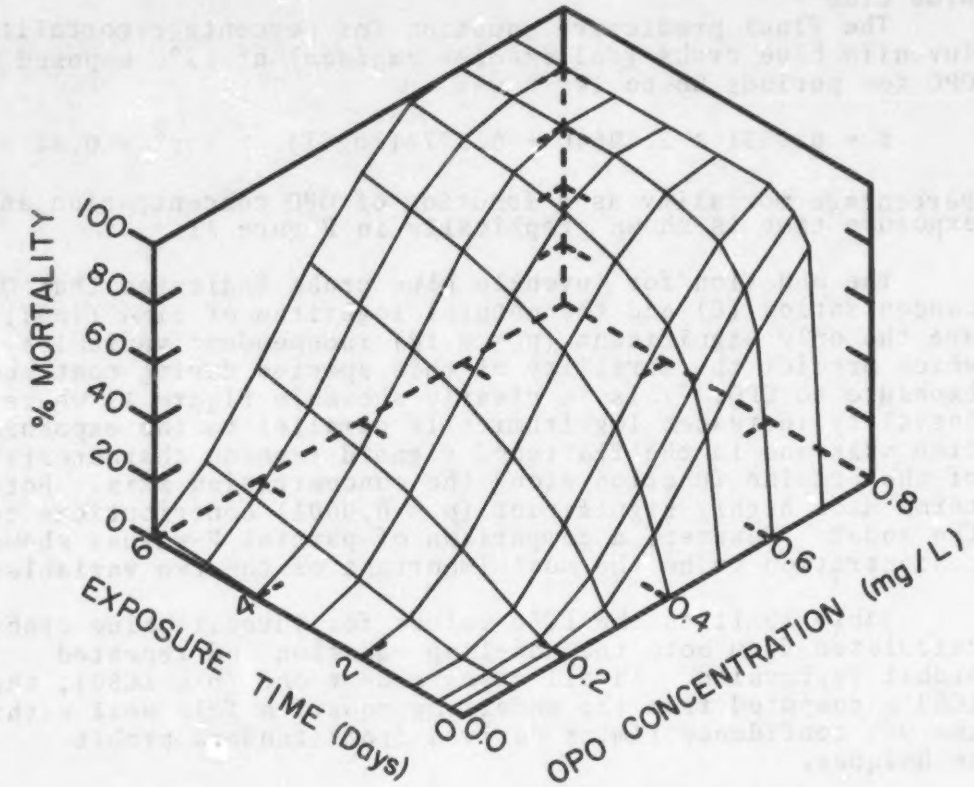

Figure 11. Predicted percentage mortality response surface for juvenile blue crabs exposed to ozone-produced oxidants (OPO) up to 6 d.

OPO's in this study at the same exposure periods (Table 15).

TABLE 15. PREDICTED LC50 VALUES CALCULATED FROM THE MORTALITY MODEL AND REPEATED PROBIT ANALYSIS FOR THE TOXICITY OF OZONE-PRODUCED OXIDANTS TO JUVENILE BLUE CRABS

Exposure Time (h)	Predicted LC50 Model	Predicted LC50 Probit	95% Confidence Limits*
6	0.53	0.63	0.590-0.698
12	0.47	0.48	0.452-0.513
18	0.43	0.43	0.350-0.508
24	0.41	0.39	0.339-0.450
36	0.37	0.36	0.306-0.437
48	0.35	0.35	0.276-0.449
72	0.31	0.30	0.269-0.321
96	0.28	0.26	0.160-0.308
120	0.26	†	† †
144	0.25	†	† †

* For probit analysis only.
† Insufficient data for analysis.

In contrast to the relatively high resistance of juvenile and adult blue crabs exposed to oxidants, Toner and Brooks (1975) found that crab zoea and megalops (species not given) were quite sensitive to OPO. All zoea exposed to 0.08 to 0.65 mg OPO/L for 10 min died 24 h after exposure. A 2-min exposure to 0.2 mg OPO/L killed 100% of the megalops 24 h after exposure. Based on Toner and Brooks' study, it appears that the larval stages of the blue crab may be much more sensitive than the juvenile and adults. Studies should be performed, however, to document this speculation for larval blue crabs.

Striped Bass--

Eggs--Predictive percent mortality models for estuarine and fresh water striped bass eggs are shown in Table 16. In both equations, OPO concentration was an important factor contributing to mortality; exposure time was not significant. Eggs tested in estuarine water had a 12- to 30-h LC50 value of 0.21 mg OPO/L when calculated by predictive equation 1. Eggs were found to be significantly ($P < 0.001$) more sensitive after 12- and 30-h exposures in fresh water than in estuarine water (Table 17). An LC50 value of 0.06 mg OPO/L (95% confidence limits; 0.03 to 0.12) was found at both exposure times in fresh water, using the probit procedure. A similar LC50 of 0.05 mg OPO/L was calculated from equation 2 in Table 16. The difference in toxicity of ozone in fresh water and estuarine water is most likely caused by different residuals that remain after ozonation. Ozone reacts quickly in estuarine water to form several oxidant species (e.g., oxidizing bromine compounds) which appear to be less toxic than ozone in fresh water, which tends to remain longer in the more reactive molecular phase (Kosak-Channing and Helz, 1979). Ozone in fresh water, however, was found in this study to decay much faster than OPO in estuarine water at the same temperature.

Delayed hatching was observed for striped bass eggs at 0.05 and 0.10 mg OPO/L in estuarine water relative to fresh water. In the fresh water study approximately 70% of the viable eggs hatched at the expected time (≈ 48 h after fertilization) at 0.05 and 0.10 mg OPO/L. However in estuarine water the following percent hatch occurred for estuarine eggs at each of the following nominal OPO concentrations: 100% at 0.0 mg/L; 100% at 0.005 mg/L; 73% at 0.01 mg/L; 8% at 0.05 mg/L and 7% at 0.10 mg/L. It should be noted that in most cases where delayed hatch occurred, embryos were still alive within the chorion. No obvious explanation for this delayed hatching can be given at the present time.

Middaugh et al. (1977a) reported 0, 3.5 and 23% hatch for estuarine striped bass eggs continuously exposed for approximately 40 h to 0.21, 0.07 and 0.01 mg CPO/L, respectively. In our investigation we found 7% hatch for estuarine striped bass eggs exposed to 0.10 mg OPO/L for approximately the same period of time. It appears in each study that CPO and OPO have a similar effect on percent hatch.

Previous investigators have reported 24- and 48-h LC50's of 0.36 and 0.20-0.22 mg CPO/L, respectively, for estuarine striped bass eggs (Morgan and Prince, 1977). The 12- to 30-h LC50 of 0.21 mg OPO/L found in our study is lower than the 24-h LC50 of Morgan and Prince (1977) but is similar to their 48-h LC50. Morgan and Prince (1978) did find that CPO concentrations of 0.05 mg/L had no significant effect on the percentage hatch for this species, however,

TABLE 16. PREDICTIVE PERCENTAGE MORTALITY MODELS AND RESPECTIVE ANOVA DATA FOR EACH STRIPED BASS LIFE STAGE EXPOSED TO OZONE-PRODUCED OXIDANTS.

Estuarine Eggs: $Y = [\sin(0.20 + 13.15C^2)]^2$ $r^2 = 0.80$ (1)

Source	df*	MS†	F§	Pψ
Regression	1	0.077	2.43	0.13
Error	28	0.031		

Fresh Water Eggs: $Y = [\sin(0.22 - 102.73C + 116.66 \log(C + 1))]^2$ $r^2 = 0.84$ (2)

Source	df*	MS†	F§	Pψ
Regression	2	1.597	80.42	0.0001
Error	27	0.028		

Larvae: $Y = [\sin(0.005 + 1263.60C - 502.50C^2 + 0.056 \log T - 1267.38 \log(C + 1))]^2$ $r = 0.93$ (3)

Source	df*	MS†	F§	Pψ
Regression	4	6.099	201.32	0.0001
Error	58	0.030		

Fingerlings: $Y = [\sin(-0.36 - 7.50C^2 + 0.12 \log(t) + 8.33 \log(C + 1))]^2$ $r^2 = 0.87$ (4)

Source	df*	MS†	F§	Pψ
Regression	3	6.624	110.28	0.0001
Error	50	0.060		

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* Degrees of freedom.
† Mean square.
§ F- statistic.
ψ Significance level.

percentage hatch for estuarine eggs was affected at 0.05 mg OPO/L in this study.

TABLE 17. ANOVA STATISTICS COMPARING THE SENSITIVITY OF FRESH WATER- AND ESTUARINE-ADAPTED STRIPED BASS EGGS EXPOSED TO OZONE-PRODUCED OXIDANTS

Source	df[*]	MS[†]	F[§]	P[ψ]
Water Type	4	0.683	26.53	0.001
Error	52	0.026		

[*] Degrees of freedom.
[†] Mean Square.
[§] F-statistic.
[ψ] Significance level.

Burton et al. (1979a and b) examined the effects of interactions between chlorine (0.00, 0.15 and 0.30 mg CPO/L), ΔT (2, 6 and 10°C) and exposure duration (0.08, 2 and 4 h) on estuarine striped bass eggs. These investigators found that TRC and exposure duration were the dominant factors resulting in mortality of the test organisms. In a univariate chlorine study, Hall et al. (in press d) found less than 6% mortality in estuarine striped bass eggs exposed to CPO concentrations of 0.06 mg/L for 12 h and 100% mortality with 36-h exposures to the same concentration. Exposure time was not found to be an important variable in the present study for either fresh or estuarine eggs (see equations 1 and 2 in Table 16). The explanation for the difference in exposure time is not clear; however, it may be related to different egg stocks or water quality conditions present during the studies.

Comparisons of data collected in this study with previously published data indicate that the effects of ozone on estuarine striped bass eggs are similar to that of chlorine. Since toxicity data on striped bass eggs exposed to chlorine in freshwater water were not available, comparisons with ozone toxicity in fresh water were not possible. The higher sensitivity found for striped bass eggs in fresh water suggests that ozone could have a more pronounced effect if discharged in fresh water industrial or municipal wastewaters located near a striped bass spawning area.

Larvae and fingerlings--Predictive percentage mortality equations and response surfaces for estuarine striped bass larvae and fingerlings are shown in Table 16 and Figures 12 and 13, respectively. Ozone-produced oxidant concentration and exposure time contributed to mortality in both life stages. The LC50 values for the larvae ranged from 0.15 mg OPO/L (95% confidence limits undefined) at 6 h to 0.08 mg OPO/L (95% confidence limits; 0.05 to 0.11) at 96 h. Fingerlings had LC50's of 0.20 mg OPO/L (0.13 to 0.27) at 6 h and 0.08 mg OPO/L (0.05 to 0.13) at 96 h. Mortality increased over time but tended to stabilize at the longer exposure periods for both life stages.

Middaugh et al. (1977a) reported 48-h LC50's of 0.04 and 0.07 mg CPO/L for 2- and 12-d old estuarine striped bass larvae, respectively. In our investigation we found a 48-h LC50 of 0.09 mg OPO/L (0.07-0.12) for this developmental stage. Comparison of the data from these two studies shows that larvae exhibit a similar response to both biocides.

Morgan and Prince (1977) reported 24-h LC50's of 0.20 and 0.19 mg CPO/L for 24- and 70-h old estuarine striped bass larvae, respectively. The 24-h LC50 for larvae in our study was 0.11 mg OPO/L (0.04-0.20). Since our 95% confidence limits overlapped the LC50's found by Morgan and Prince, it appears that the effects from each oxidant are similar.

Interactions between chlorine (0.00, 0.15 and 0.30 mg CPO/L), ΔT (2, 6 and 10°C) and exposure duration (0.08, 2 and 4 h) have been studied by Burton et al. (1979a and b) with 24-h old estuarine striped bass prolarvae. Total residual chlorine, ΔT and exposure time were all found to be important factors resulting in mortality of this life stage. Precise comparisons between the Burton et al. studies and the present investigation are difficult due to different testing procedures. However, Burton et al. found 100% mortality after 4 h of exposure at a concentration of 0.15 mg CPO/L and a ΔT of 0°C, while in the present study, we found a 6-h LC50 of approximately 0.15 mg OPO/L (95% confidence limits undefined). The differences in larval mortality between studies could be due to the age of the test organisms.

Previous investigations in our laboratory have shown that approximately 75% mortality occurred in estuarine striped bass larvae (7 to 9 mm) subjected to 0.06 mg CPO/L for 96 h (Hall et al., in press d). In the present study we found a 96-h LC50 of 0.08 mg OPO/L (0.05 to 0.11) for striped bass larvae. A comparison between these studies suggest that larvae may be slightly more sensitive to CPO than OPO.

A comparison between the ozone toxicity data from this study and the chlorine toxicity literature for striped bass

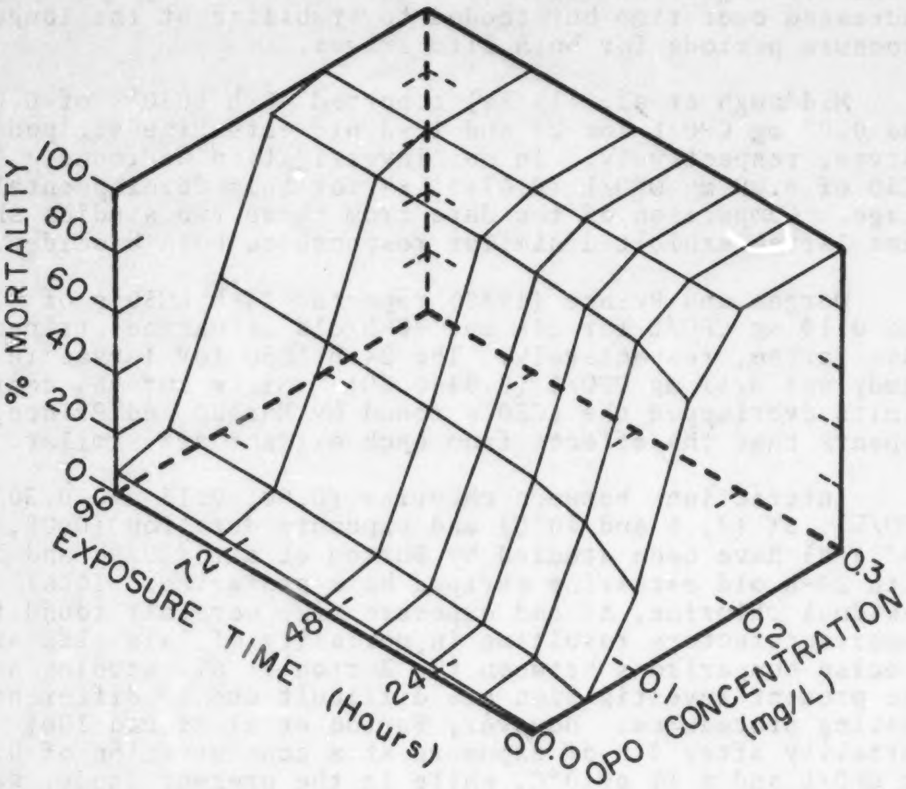


Figure 12. Predicted percentage mortality response surface for striped bass larvae exposed to ozone-produced oxidants (OPO) up to 96 h.

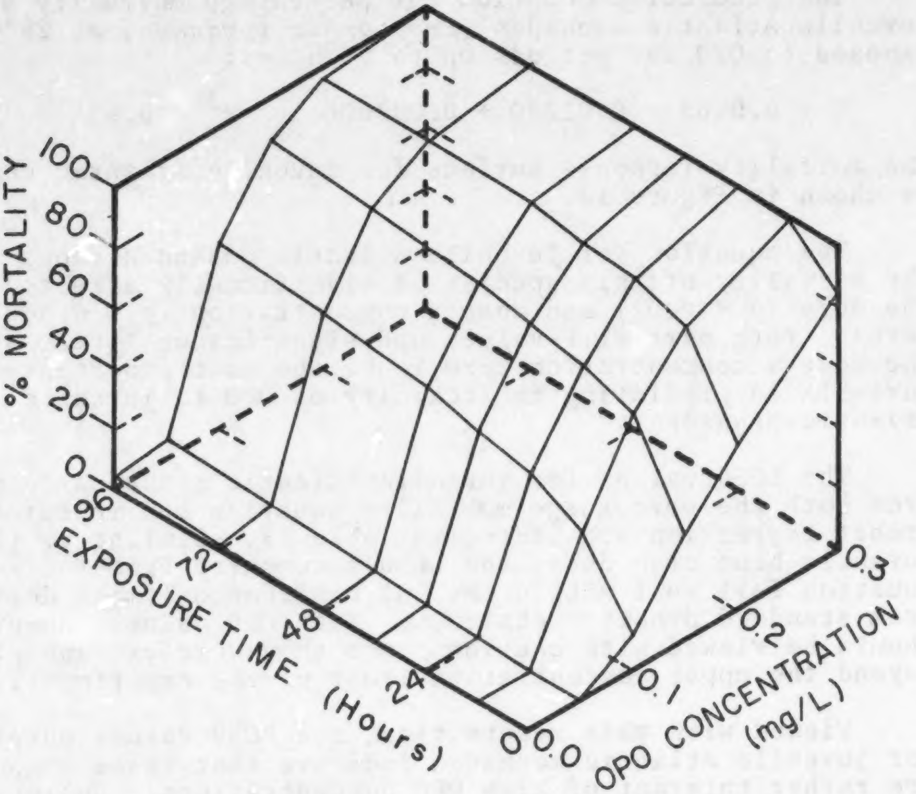


Figure 13. Predicted percentage mortality response surface for striped bass fingerlings exposed to ozone-produced oxidants (OPO) up to 96 h.

larvae indicates that the toxicity of ozone and chlorine to this species is similar in estuarine water. This is not surprising since our bromate studies and several other recent studies indicate that the chemical reaction pathways and mechanisms may be similar with these two biocides (see review by Helz, in press).

Atlantic Menhaden--

The predictive equation for percentage mortality of juvenile Atlantic menhaden (*Brevoortia tyrannus*) at 25°C exposed to OPO for periods up to 96 h was:

$$Y = 0.0063 - 0.0122D + 0.0976DC \qquad r^2 = 0.93$$

The mortality response surface for juvenile Atlantic menhaden is shown in Figure 14.

The equation for juvenile Atlantic menhaden shows that the mortality of this species is significantly affected by the dose ($p < 0.02$) and dose x concentration ($p < 0.0001$) terms. Both partial F-values and significance levels proved the dose x concentration term to be the most important variable in predicting the toxicity of OPO to juvenile Atlantic menhaden.

The LC50 values for juvenile Atlantic menhaden calculated from both the percentage mortality equation and repeated probit regression are listed in Table 18. Similar to the juvenile blue crab data, the LC50's computed from the modeling equation fell well within the 95% confidence limits derived from standard probit techniques. The LC50 values, however, should be viewed with caution since they were extrapolated beyond the upper concentration limit of the experiment.

Viewed with this reservation, the LC50 values obtained for juvenile Atlantic menhaden indicate that these organisms are rather tolerant of high OPO concentrations. Gullens et al. (1977) established a 96-h LC50 of 0.15 mg/L for 25°C acclimated Atlantic menhaden exposed to CPO. This is approximately half the 96-h value found for the fish exposed to OPO in this study and suggests that the values obtained in this study are higher than one would expect. Data for other species in this study when compared to CPO values in the literature for the same species indicate that similar concentrations of OPO and CPO produce similar effects.

White perch--

Acute studies--The final predictive equation selected by the stepwise analysis for the mortality of adult white perch (*Morone americana*) exposed to OPO at 15°C for 96 h was:

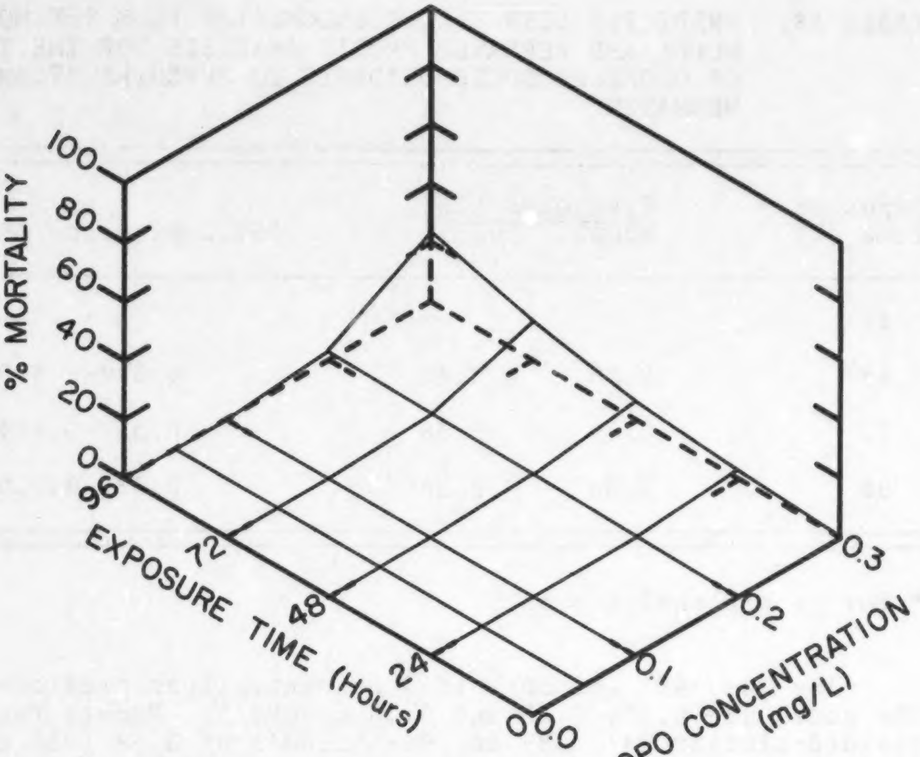


Figure 14. Predicted percentage mortality response surface for juvenile Atlantic menhaden exposed to ozone-produced oxidants (OPO) up to 96 h.

$$Y = 0.2908 - 0.0096(T) + 0.1690(D) - 0.25(D)^2 \qquad r^2 = 0.85$$

Partial F-statistics showed dose to be the single most important variable in the equation. Exposure time was slightly more important than OPO concentration, as indicated by the inclusion of a time variable in the final model. However, as demonstrated in Figure 15, both factors contributed almost equally in producing a given mortality response.

TABLE 18. PREDICTED LC50 VALUES CALCULATED FROM THE MORTALITY MODEL AND REPEATED PROBIT ANALYSIS FOR THE TOXICITY OF OZONE-PRODUCED OXIDANTS TO JUVENILE ATLANTIC MENHADEN

Exposure Time (h)	Predicted LC50		95% Confidence Limits*
	Model	Probit	
24	-	-	-
48	0.48	0.42	0.370-0.590
72	0.40	0.38	0.351-0.464
96	0.36	0.36	0.335-0.400

* For probit analysis only.

The 24-, 48- and 96-h LC50 concentrations predicted by the model are 0.37, 0.23 and 0.16 mg OPO/L. Probit regression yielded similar 24-, 48- and 96-h LC50's of 0.38 (95% confidence limits; 0.352 to 0.420), 0.26 (0.236 to 0.268) and 0.20 (0.181 to 0.223) mg OPO/L, respectively. None of the fish exposed to OPO concentrations ≤ 0.10 mg/L lost equilibrium or died during the 96-h exposure period. However, most of the white perch exposed to ≈ 0.10 mg OPO/L were extremely lethargic and insensitive to tactile stimulation. At concentrations > 0.10 mg OPO/L fish always experienced a period of loss of equilibrium before death.

The toxicity of OPO to several estuarine organisms has been shown in this study and the study by Block et al. (1978) to be similar to CPO toxicity. Gullans et al. (1977) reported the 96-h LC50 for adult white perch exposed to CPO at 15°C to be 0.21 mg/L compared to that of 0.20 mg CPO/L from this study. Both studies were conducted at the same

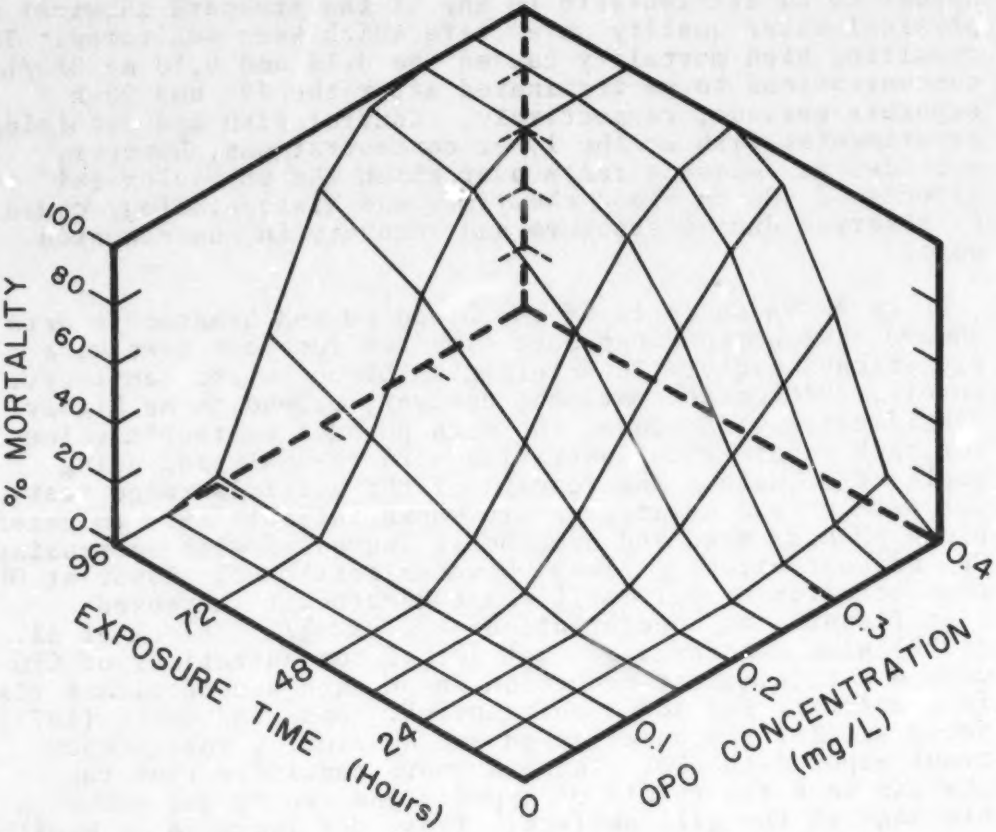


Figure 15. Predicted percentage mortality response surface for adult white perch exposed to ozone-produced oxidants (OPO) up to 96 h.

laboratory under similar water quality conditions.

Exposure and recovery studies--These studies were conducted at OPO concentrations near or below the 96-h LC50 estimated from the acute toxicity studies. However, the toxicity of the same amperometrically measured OPO concentrations was slightly greater than that found during the previous acute study. These toxicity differences do not appear to be attributable to any of the standard chemical or physical water quality parameters which were monitored. The resulting high mortality caused the 0.15 and 0.10 mg OPO/L concentrations to be terminated after the 48- and 96-h exposure periods, respectively. Control fish and remaining experimental fish at the lower concentrations, however, provided an adequate range over which the physiological effects of OPO on blood chemistry and histopathology could be observed during exposure and recovery in non-ozonated water.

An ANOVA analysis of the blood pH and hematocrit data showed that neither exposure time nor recovery time were significant factors in determining blood pH and hematocrit levels. OPO concentrations, however, proved to be highly significant. Therefore, the mean pH and hematocrit values for each exposure concentration were re-analyzed, using pooled time data. The results of the multiple range test for both pH and hematocrit are shown in Table 19. In general, blood pH decreased and hematocrit increased with increasing OPO concentrations. Blood pH was significantly lower at OPO concentrations > 0.10 mg/L while hematocrit increased significantly at concentrations > 0.05 mg/L. Block et al. (1978) also demonstrated that lethal concentrations of CPO caused a significant reduction in pH with a concomitant rise in hematocrit for adult white perch. Bass and Heath (1977) found similar responses in pH and hematocrit for rainbow trout exposed to CPO. These authors concluded that the changes were the result of hypoxia induced by gas exchange blockage at the gill surface. Thus, the increase in hematocrit appears to be a physiological compensation to increase the oxygen-carrying capacity of the blood. The reduction in pH may be caused by a buildup of CO_2 in the blood and a partial shift to anaerobic metabolism.

Gill histology was extremely useful in clarifying the physical mechanism which induced mortality of adult white perch exposed to OPO. Figure 16 shows the progressive tissue destruction caused by increasing OPO concentrations. Plate A (Figure 16) shows a normal gill filament. Slight membrane separations are artifacts produced during tissue preparation. Plate B was taken from a fish exposed to 0.01 mg OPO/L for 96 h. At this concentration, there is little apparent tissue damage, no physiological changes occurred

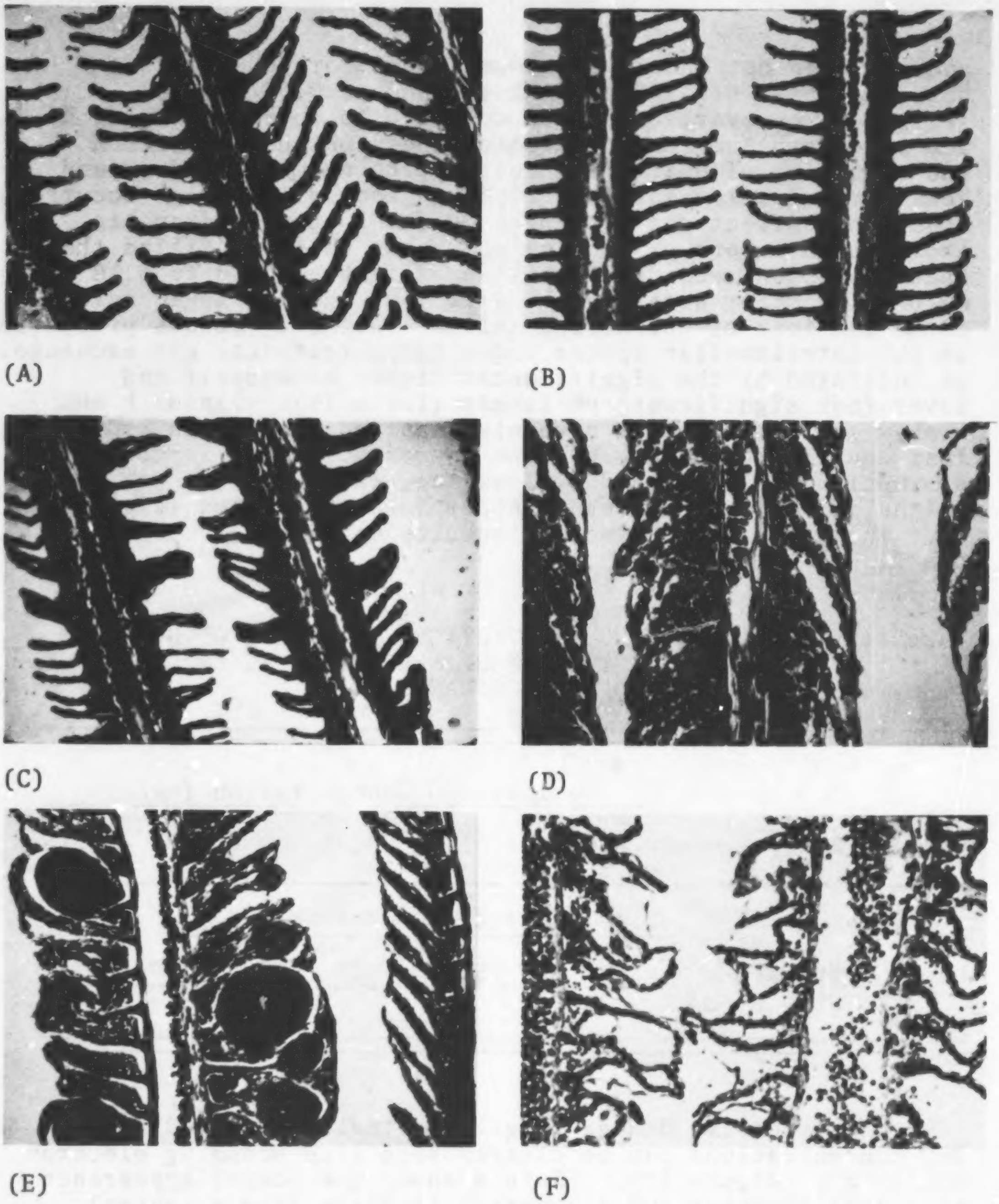


Figure 16. Sagittal sections of the primary and secondary gill lamellae from white perch exposed to A) control conditions (x125); B) 0.01 mg OPO/L for 96 h (x125); C) 0.05 mg OPO/L for 96 h (x125); D) 0.10 mg OPO/L for 48 h (x300); E & F) 0.15 mg OPO/L for 48 h (x300).

and fish did not show any abnormal behavior (qualitative observation). Gills from fish exposed to 0.05 mg OPO/L (Plate C), however, exhibited moderate to severe clumping of the secondary lamellae with some excess mucous production and sloughing of epithelial cells. The normal blood pH and hematocrit levels show that although some gill damage occurred, it did not affect gas exchange. Plate D shows a dramatic increase in mucous production which has entirely filled the spaces between secondary lamellae of fish exposed to 0.10 mg OPO/L. Other areas of the gill (not shown) showed severe edema and loss of epithelial cells. The heavy mucous production in the interlamellar spaces undoubtedly restricts gas exchange, as indicated by the significantly higher hematocrit and lower (not significant) pH levels (Table 19). Plates E and F show gills taken from severely impaired fish which had lost equilibrium and were close to death. There is complete disruption of the secondary lamellae, extensive loss of epithelial tissue and severe hyperplasia and edema (Plate E). This massive destruction results in eventual death of the fish.

TABLE 19. MULTIPLE RANGE STATISTICS ($p < 0.05$) OF BLOOD pH AND HEMATOCRIT CHANGE IN ADULT WHITE PERCH EXPOSED TO OZONE-PRODUCED OXIDANTS (OPO)

	Nominal OPO Concentration (mg/L)				
Blood Parameter	0.00	0.01	0.05	0.10	0.15
pH	7.47	7.47	7.47	7.31	7.19
Hematocrit (%)	23	23	27	38	39

The extensive damage to gill epithelium exposed to high OPO concentrations can be clearly seen with scanning electron microscopy (Figure 17). Plate A shows the normal appearance of a gill filament and associated lamellae from a control fish. The squamous epithelial cells are very smooth and give the lamellae a regular appearance. Intercellular boundaries are marked by a relatively inconspicuous double ridge. Plate B (from the initial LC50 study) shows the effect of 0.30 mg OPO/L on the epithelium and structural integrity of the secondary lamellae. The secondary lamellae are broken in many areas and have an extremely irregular

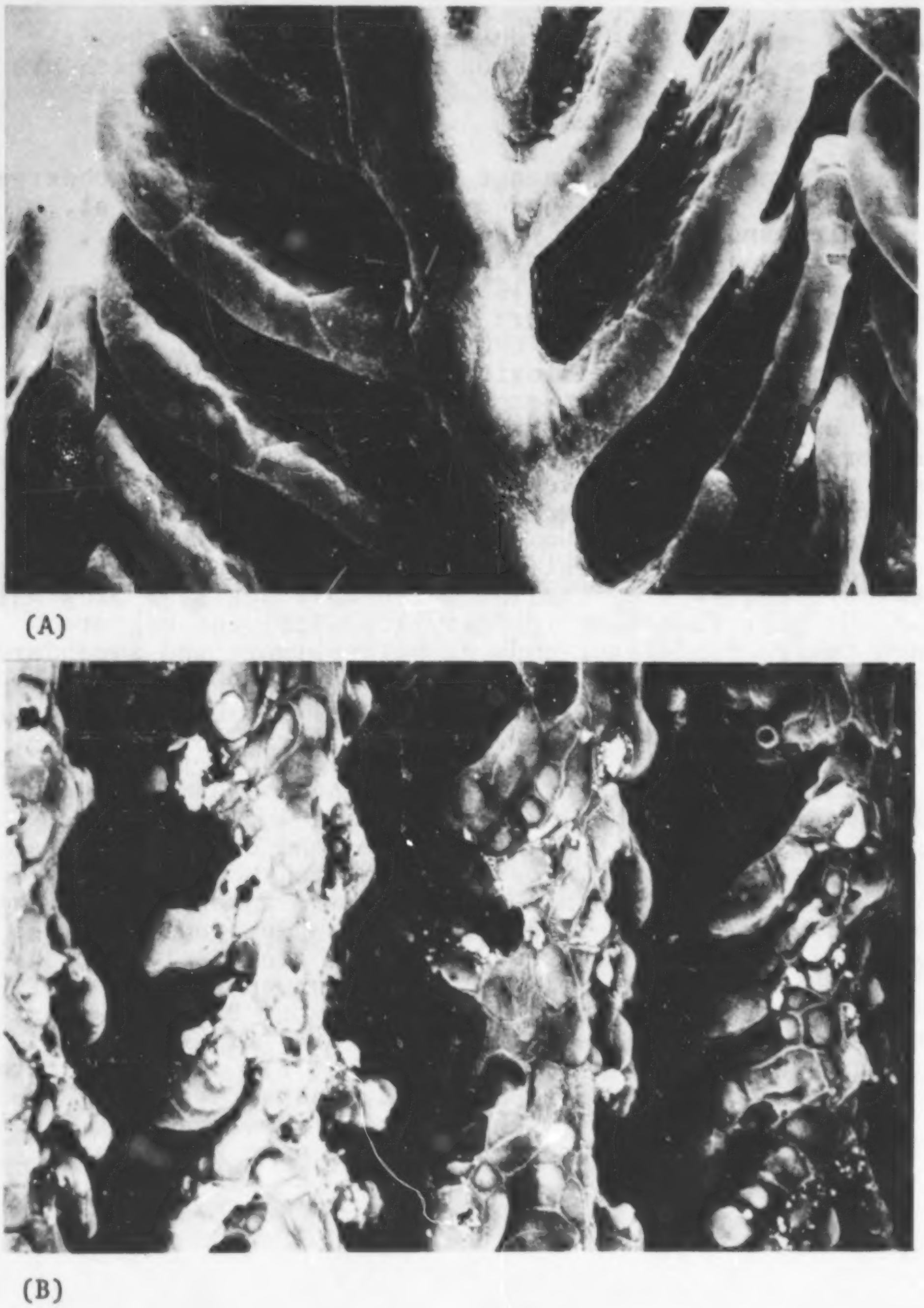
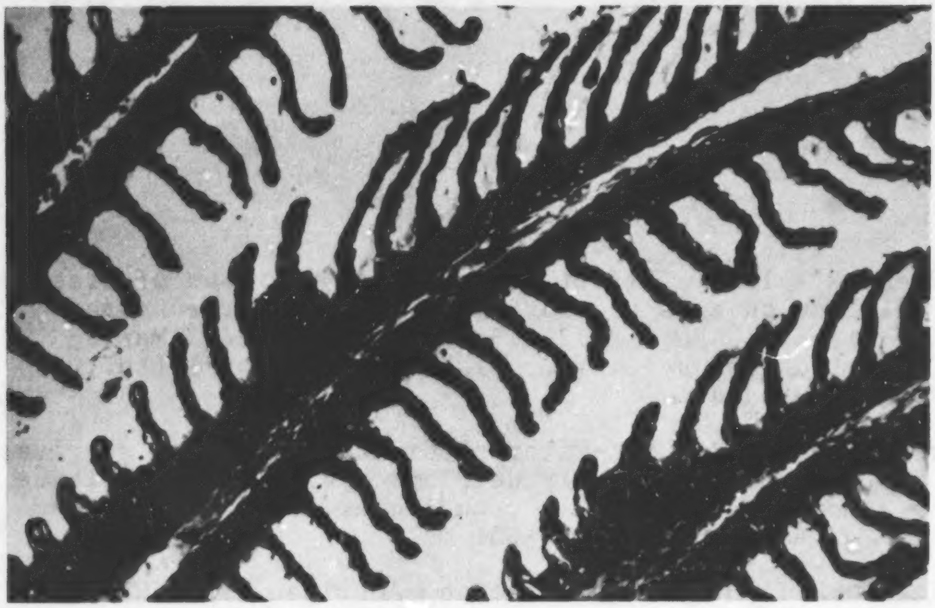


Figure 17. Scanning electron micrographs of primary and secondary gill lamellae from white perch exposed to A) control conditions (x1000); B) 0.30 mg OPO/L until death (x500).

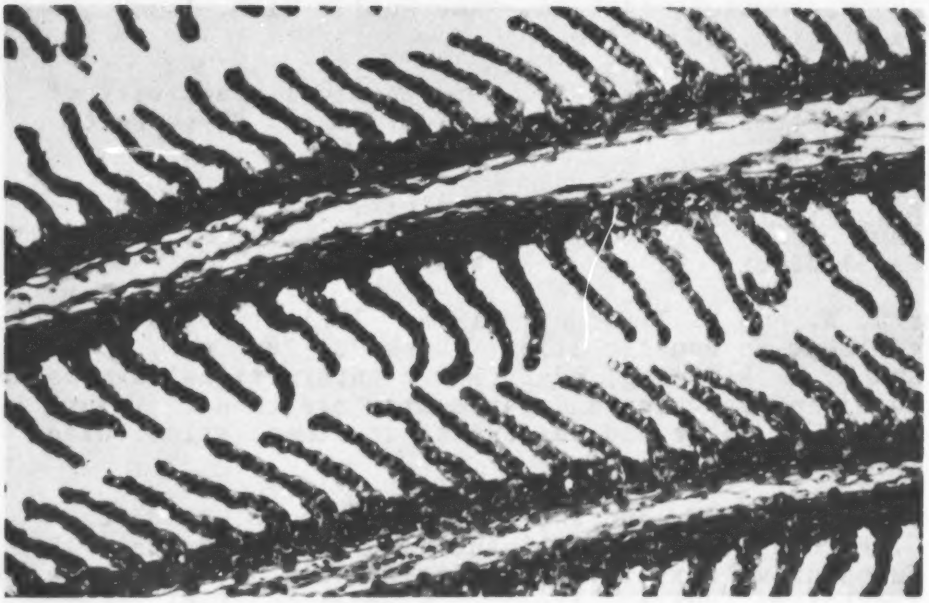
appearance. Many sites of ruptured or missing epithelial cells can be seen. The squamous epithelial cells which are still intact appear shrunken, making the intercellular boundaries quite pronounced.

These types of gill damage have been previously observed for freshwater teleosts exposed to OPO (Wedemeyer et al., 1979; Paller and Heidinger, 1980) and CPO (Bass et al., 1977) and for an estuarine fish (spot) exposed to CPO (Middaugh et al., 1977b and 1980). Little work has been done, however, on the recovery potential of fish surviving moderate to high dosages of OPO or CPO. Bass and Heath (1977) examined the physiological and histopathological responses of rainbow trout exposed to 2-h pulses of ≈ 0.5 mg CPO/L every 8 h. They found that the effects of each pulse were cumulative with only minor physiological recovery between the first few pulses. Thus, damage done to the gills of rainbow trout (*Salmo gairdneri*) requires more than 6 h to repair. MacKinnon and Enesco (1980) have shown that the epithelial cells of teleost fish constitute a cell renewal system. New epithelial cells form near the proximal end of the gill filaments (primary lamellae) and migrate outward toward the distal ends of both primary and secondary lamellae. The migration rate for the rosy barb (*Barbus conchonius*) was approximately 1.2 μm/h with a projected epithelial replacement rate of about 100 h.

Based upon this replacement rate, severe damage to the gills of white perch should take at least 4 d to repair, assuming the underlying basal membranes and columnar cells are relatively intact. In our experiments, no fish exposed to concentrations ≥ 0.10 mg OPO/L survived through the first 4 d recovery sampling period. This was undoubtedly because the gill tissue could not repair itself before restrictions of the respiratory and osmoregulatory systems proved fatal. Gills from fish exposed to 0.05 mg OPO/L for 96 h, however, showed almost complete recovery within 4 d (Figure 18; A) and complete recovery in 14 d (Figure 18; B). This indicates that the replacement rate in the cell renewal system of the gill epithelium in white perch is of the same order of magnitude as that of the rosy barb (MacKinnon and Enesco, 1980).



(A)



(B)

Figure 18. Sagittal sections of primary and secondary lamellae from adult white perch exposed to 0.05 mg OPO/L for 96 h and then allowed to recover for A) 4 d; B) 14 d (A & B x125).

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X 16. ABSTRACT

This research program was initiated to determine the chemical and biological effects of ozone in estuarine systems and to evaluate its suitability as an alternative to chlorine. Chemical studies were performed to determine oxidant decay rates and bromate formation in both natural and artificial estuarine water. Ozonation of estuarine or marine waters can produce significant amounts of bromate if the natural levels of ammonia-nitrogen and other competing compounds are low and the initial residual oxidant and bromide concentrations are high. Toxicity studies showed that the concentrations of bromate which theoretically could be formed in an ozonated discharge were not toxic to the early life stages of striped bass (*Morone saxatilis*) and juvenile spot (*Leiostomus xanthurus*). Acute toxicity studies of ozone-produced oxidants (OPO) showed that the early larval stages of the American oyster (*Crassostrea virginica*) and recently hatched striped bass ichthyoplankton were most sensitive to OPO; this sensitivity decreased with progressive development. Juvenile blue crabs (*Callinectes sapidus*), juvenile Atlantic menhaden (*Brevoortia tyrannus*) and adult white perch (*Morone americana*) were more resistant than molluscan and ichthyoplankton larval stages. Hematological and gill histopathological sublethal studies of adult white perch showed that OPO caused death by hypoxia as a result of excessive gill damage. Moderately damaged gill tissue repaired itself within a few days when fish were moved to clean waters. A comparison of the ozone data obtained in this study with similar chlorine data from the literature showed that the toxicity of ozonated and chlorinated estuarine water is similar. Thus, ozone appears to offer few, if any, advantages over chlorine in reducing toxicity to estuarine organisms.

17. KEY WORDS AND DOCUMENT ANALYSIS

X a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Bromate Oxidant Ozone Toxicity *Brevoortia tyrannus* *Callinectes sapidus* *Crassostrea virginica* *Leiostomus xanthurus* *Morone americana* *Morone saxatilis*	X	X

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